CE

EVE™

Automated Cell Counter

User Manual

NanoEntek

EVE™ User manual

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The information in this manual is described as accurately as possible.
Firmware and software changes and updates may change without prior consent or notification.

Documentation : **NESMU-EVE-001E**

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: V.0.5 Date : MAY 2013
: V.0.8 Date : OCT 2013
: V.1.5 Date : APR 2020
: V.1.6 Date : July 2021

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Product contents

EVE™ is shipped with the following components.
Please check that all items listed below were shipped, after receiving the instrument.
If any items are missing or damaged, contact your local distributor or e-mail sales@nanoentek.com.

Automated cell counter

1 EA

Cat. No. EVE-MC

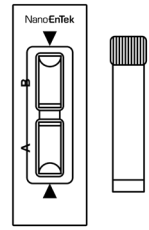
Power cord with 4 adaptor cords (4 pcs/set, for U.S./Canada/ Taiwan/Japan, Europe or UK)

1 SET

Cell counting slides

(with 1.5 mL of Trypan blue (0.4%))

1 BOX (50 slides/box)



Cat. No.
EVS-050

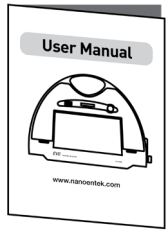
USB drive

2 GB

1 EA

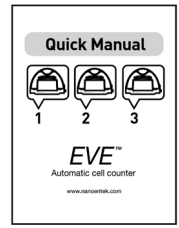
User manual

1 EA



Quick manual

1 EA



Product overview

The EVE™ Automated Cell Counter uses state-of-the-art optics and image analysis to automatic cell counting. The EVE™ is a benchtop counter designed to measure cell count and viability (live, dead, and total cells) accurately and precisely, using the standard trypan blue technique.

Using the same amount of sample that you currently use with the hemocytometer, the EVE™ takes less than 20 seconds per sample for a typical cell count and is compatible with a wide variety of eukaryotic cells and provides information on cell size.

The EVE™ offers an intuitive user interface, and provides the option to save and print cell count data using the EVE™ Software and USB drive supplied with the instrument or available separately.

The EVE™ is supplied with disposable EVE™ Cell counting slides that contain two enclosed chambers to hold the sample to allow you to measure two different samples or perform replicates of the same sample. The cell counting occurs in the central location of the counting chamber and the volume counted is 0.4 µL, the same as counting four (1 mm × 1 mm) squares in a standard hemocytometer.

Product overview

Features and benefits

—

User-friendly, benchtop design for simple, fast, automated cell count and viability measurements within **20 seconds**.

—

Provides data based on cell size and is compatible with a **wide variety** of eukaryotic cells without the need for any special changes between large and small sizes.

—

Measures cell concentrations ranging from **1 × 10^4 to 1 × 10^7 cells/mL** and cells with sizes ranging from **5 µm to 60 µm**.

—

Provides the **clump cell counting function** to get more accurate results (using EVE™ PC software).

—

Uses **disposable cell counting slides** that eliminate washing steps and cross contamination between samples.

—

Saves and prints cell count data including images using the EVE™ software and EVE™ USB drive.

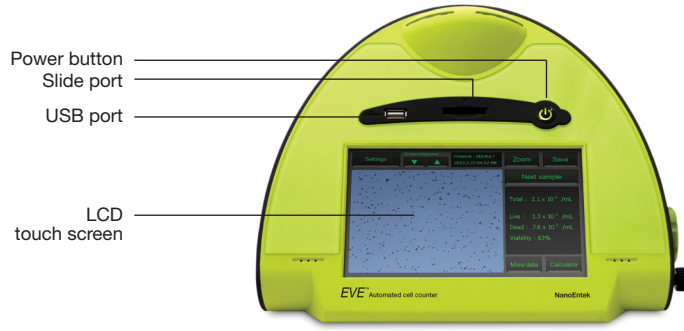
—

Presents comprehensive data with graphical reports and **as a .CSV** (comma separated value) file for sample comparisons.

Description

Front view

Power button	The power button is used to turn the instrument on and off. The red status light indicates that the instrument is off; the green status light indicates that the instrument is on.
LCD touch screen	Located in front of the instrument, LCD touch screen contains buttons for all the functions needed and displays data from the cell count.
Slide port	The slide port is used to insert the EVE™ Cell Counting Slide containing the sample with trypan blue stain into the counter for analysis.
USB port	The USB port allows you to transfer and save the cell count data and image to your computer for record keeping and printing purposes. The USB drive supplied with the instrument or any other standard USB drive is inserted into the USB port for data transfer.



[Front view]

Description

Rear and side view

Power inlet	Connect the counter to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.
Image adjustment (focus) knob	The image adjustment (focus) knob isused to adjust the image quality to obtain better contrast between live (bright centers) and dead(dark blue centers) cells. This is important to obtain accurate cell counts and viability measurements.
Focus lock knob	The focus lock knob is used to lock the image adjustment (focus) knob once the image is optimized. There is no need to use the focus lock knob , but is available for convenience, if you are measuring multiple samples of the same cell type.

[Rear view]

[Side view]

Installation

Installing EVE™ Automatic cell counter

1. After unpacking the instrument, place the instrument on a flat, level, dry surface.

2. Plug one end of the supplied power cord into the EVE™. To the other end attach the appropriate plug adaptor, based on the electrical outlet configuration in your country.

3. Plug the power cord into the electrical outlet. Be sure to use only the power cord supplied with your instrument. Powering the instrument with an unapproved power cord may damage the instrument.

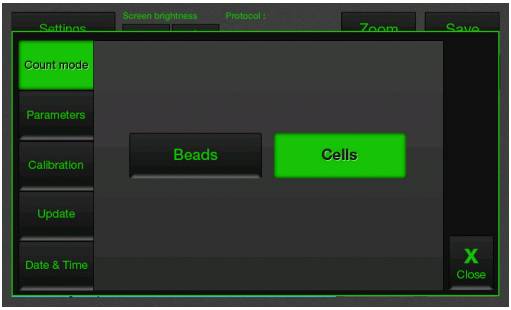
4. When you are ready to use, start the EVE™ by pressing the **Power button**.

5. When the instrument is turned on, the Start Up screen is displayed. Here you can proceed immediately to cell counting, set up the instrument for cell or bead count, or adjust the screen brightness.

Operation

Settings

1. Press **Settings** from the Start-Up screen to display Settings.



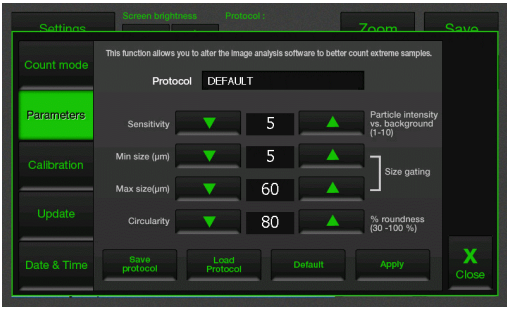
The settings menu allows you to set up the following:

· **Count mode** to operate the instrument for cell counting (choose Cells) or bead counting (choose Beads)
· **Parameters** (see below and next page for details)
· **Calibration** to calibrate the instrument (page 24)
· **Update** to install new firmware versions as they become available
· **Date and Time** to set up date and time (page 13)
· Use the scroll buttons to adjust the **screen brightness**.

Cell mode parameters

1. Press **Parameters** from the settings screen to display Cell mode parameters screen.

The Parameters function allows you to change the image analysis algorithm for specific or mixed cell types, and the specific parameters must be determined empirically.



Operation

Cell mode parameters

2. The cell mode parameters are described below:

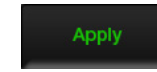
· **Sensitivity** (refers to the contrast of the objects from the background). Adjusting the sensitivity higher makes instrument more sensitive to objects; useful for cells that do not stain well with trypan blue while adjusting the sensitivity lower makes the instrument less sensitive and is useful if there is a lot of background.

· **Minimum cell size** is used to determine the low range of cell size to include in the measurement. The algorithm first identifies all objects, and calculates the average size (e.g., 15 µm). From the percent of average size setting, the algorithm calculates the smallest object size to include in the final measurement (e.g., 70% of 15 is 10.5 µm; 15–10.5 = 4.5 µm; 4.5 µm would be the smallest particle included in the count). Adjusting the number up, increases inclusiveness thereby decreasing the lower cell size range (e.g., 50% of 15 is 7.5 µm; 15–7.5 = 7.5 µm).

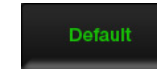
· **Maximum cell size** is used to determine the high range of cell size to include in the measurement. The algorithm first identifies all objects, then calculates the average size (e.g., 15 µm). From the percent of average size setting, the algorithm calculates the largest object size to include in the final measurement (e.g., 200%; 200% of 15 µm = 30 µm; 30 µm is the largest cell size included in the measurement.

· **Circularity** is used to determine the objects to include in the measurement based on roundness. Increasing the value from 80% requires objects to be more round for inclusion in the measurement. Decreasing the value from 80% allows objects to be less round. Adjusting this may be useful if the cell type is not particularly circular or perhaps oddly shaped.

After modifying any parameters,
press **Apply** to make the changes.



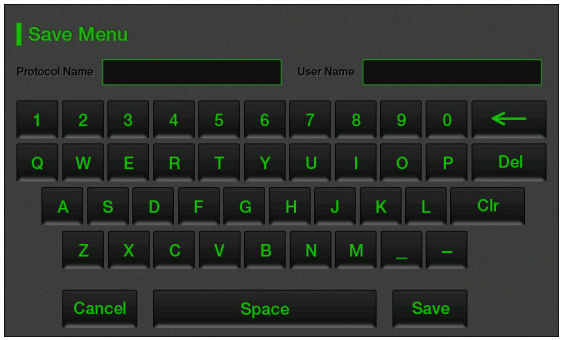
To restore default parameters, press **Default**.



3. Press **Save Protocol** to create a new protocol.

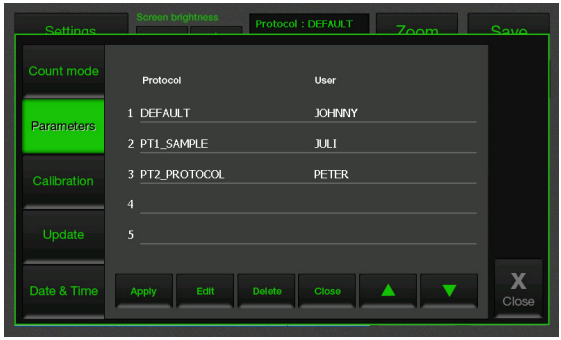
Cell mode parameters

4. Type **Protocol Name** and **User Name** in the appropriate fields, and press **Save** again.



5. Once a protocol is saved, it is available for use at any time. Press **Load Protocol**.

6. The protocol appears in the protocol menu. Use the up and down arrows to find your saved protocol.
To use the protocol, select one of protocol and press **Apply**.

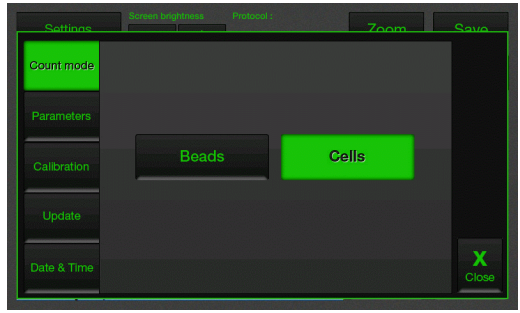


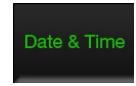
» press **Delete** to delete protocol.
press **Edit** to edit protocol.

Date and time setup

1. Turn on the EVE™ by pressing the **Power button**. The Start up screen is displayed after a few seconds.

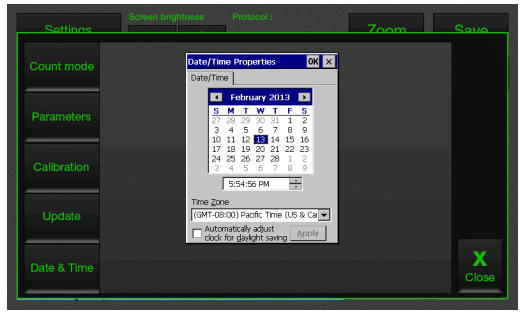
2. Press **Settings** and then press **Date and Time**.





3. The **Date/Time Properties** screen is displayed. To select the month and day, scroll to the appropriate month using the arrow keys and then press the day on the calendar.

» Use a pointed object, like a stylus or pipette tip, to push the small buttons on the calendar.



4. To select the time, scroll to the appropriate time and select **Automatically adjust clock for daylight saving**, if necessary. Press **Apply** or **OK** to make the date/time changes. The updated date/time is displayed on the top of the window. Once the date/time is set, there is no need to set it each time the instrument is turned on.

5. Press **Close** to exit the screen.

Operation

Recommend actions

To obtain the best results, follow these recommendations:

· Wear Protective gloves during sample handling.

· Do not touch the optical surfaces of the EVE™ Cell counting slides. Hold the cell counting slides by the edges.

· Use the EVE™ at room temperature only (5 - 40 °C).

· For accurate viability count results, ensure the counting area is covered with cell suspension and count cells within 3 minutes of mixing the cells with trypan blue solution as trypan blue is toxic to cells. For best data with biological samples, we recommend counting at least two samples and taking an average.

· The EVE™ is supplied pre-calibrated. To recalibrate your instrument, see page 24.

· The EVE™ memory holds one set of data. Save your data to the USB drive after each reading. You may transfer the data to your PC, using the USB drive immediately as described in **Transferring data to a PC** (page 21).

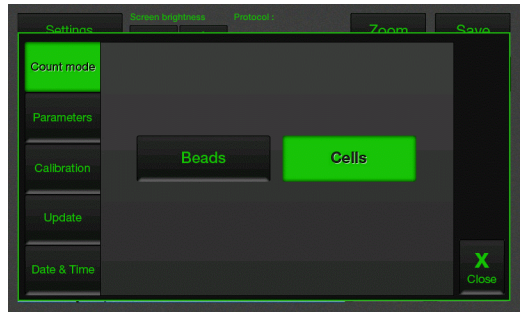
· After using EVE™, appropriately dispose slides as biohazardous waste. **Do not reuse the cell counting slides**.

Operation

Cell counting

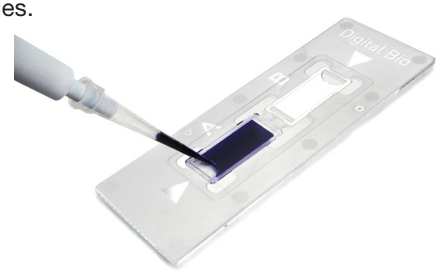
1. Push the **Power button** to start the instrument. The Start-up screen is displayed.

2. The EVE™ is preset to **Cells mode**.



3. Mix well the 10 µL of your sample and the 10ul of 0.4% trypan blue stain (in a 1:1 ratio) using a pipette.

4. Load 10 µL of the sample mixture on EVE™ Cell counting slide (side A) using a pipette.
The two chambers of the slide are labeled “A” and “B” for easy tracking of your samples.

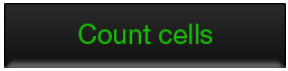


Operation

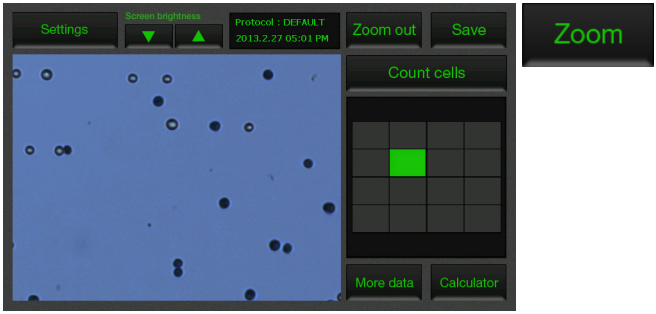
Cell counting

5. Insert the EVE™ Cell counting slide, sample side (side A) first into the slide port on the instrument, you will hear a soft click, if the slide is pushed in correctly. Each chamber is counted separately.

6. Press the **Count cells** or **Next sample button**.

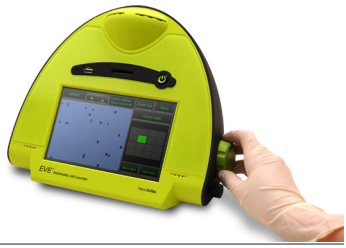


7. Adjust the image by pressing the **Zoom button**. Navigate by pressing the location you like to see on the grid.



8. While viewing cells in the zoom mode, use the **Focus knob** to adjust the image.

» After you have counted the first sample, you may not have to use the Focus knob again. If measuring multiple samples of the same approximate size, you may lock the Focus knob. You are able to unlock the knob later, to adjust the image.



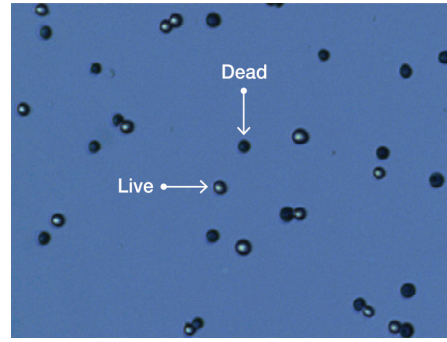
Operation

Cell counting

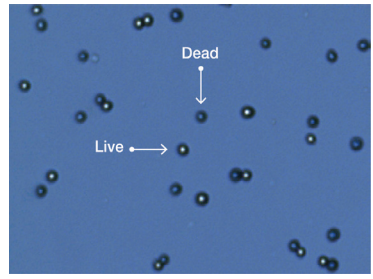
Optimize the image for analysis such that:

- **Live cells** have bright centers and dark edges.

- **Dead cells** have a uniform blue color throughout the cell with no bright centers.

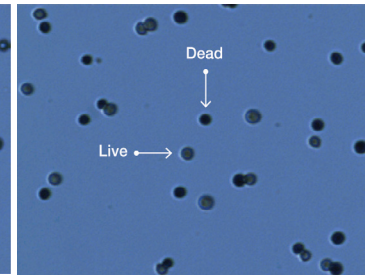


<Correct Image>



<Incorrect Image 1>

Dead cells have bright, blue centers are counted as the live cells.

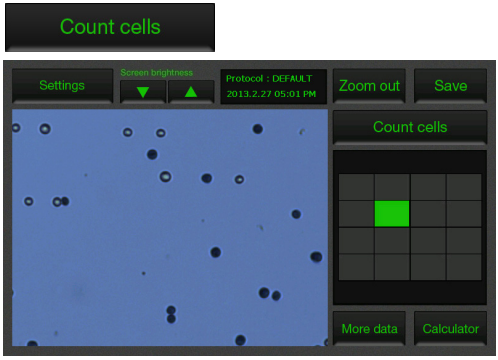


<Incorrect Image 2>

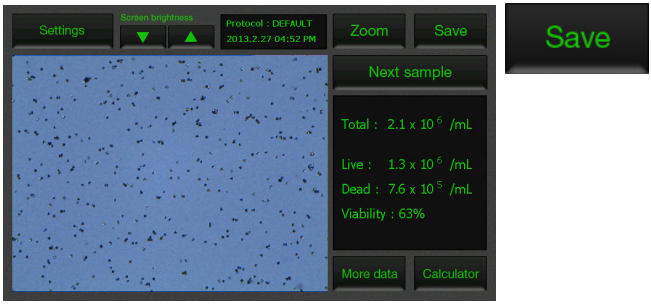
Live cells have dark centers and are counted as the dead cells.

Cell counting

9. When you are satisfied with the image, press **Count Cells**.



10. After 20 seconds to count each sample, and the cell count for live, dead, and total cells as well as percentage viability is displayed on the screen. Record the cell count, or insert a USB drive and press **Save button** (see page 20).



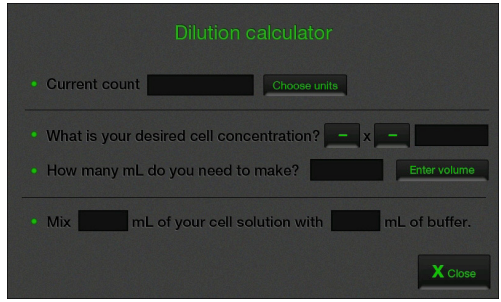
11. To see more details on the data as well as graphical representation of the data, press the **More data button**.

» Press the **Close button** to return to the main screen.

Operation

Cell counting

12. The **Calculator button** allows you to quickly calculate adjustments to the cell suspension to obtain a desired concentration.



13. To count the cells in the other side of the slide (side B), remove the EVE™ slide after side A is counted by pushing in the slide slightly and then pulling the slide out. Turn the slide around and reinsert into the slide inlet and repeat the procedure.

14. The EVE™ USB drive holds one set of data. To save your data for future analysis or archiving, you must record the data or save after each reading.
See page 21 for **Transferring data to a PC**.

15. After recording or saving the data, remove and discard the slide appropriately as bio-hazardous waste.

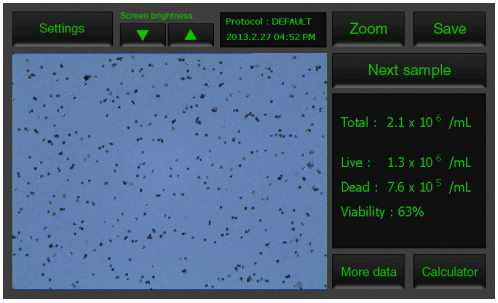
16. At this point, the EVE™ is ready for another sample. If you are not using the instrument, press the **Power button** to turn off the instrument.

» If the touch screen is not responding, you can turn off the instrument by pressing and holding the **Power button** for 4 seconds.

Transferring data to a computer

1. To archive your data or generate a printed report, insert EVE™ USB drive into the USB port.

2. Save your data on the USB drive by pressing the **Save button** on the main screen. The image and the counting data are saved.



3. Enter the file name using the keypad buttons displayed on the Save menu.

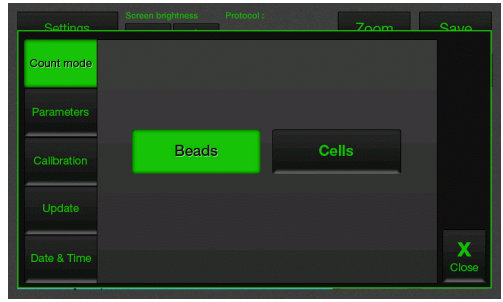
» The numerical data is also automatically saved as a .CSV file that can be opened with any spreadsheet program. To delete all data from the .CSV file and start with a blank file, press **Start .CSV file button**.

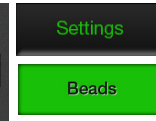
4. Transfer the EVE™ USB drive to the USB port on your PC. You may open the .CSV file using a spreadsheet program. To see the image and generate a report, use the EVE™ PC software (on page 25).

Operation

Using the beads mode for counting beads

1. Press **Settings** and then press **Beads** to place the instrument into bead counting mode.



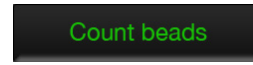


2. Put 10 µL of beads to 10 µL of 0.4% trypan blue stain, (in a 1:1 ratio) and mix well. Mix gently by pipetting up and down.

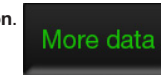
3. Load 10 µL of the sample mixture on EVE™ Cell counting slide (side A or B) using pipette. The two chambers of the slide are labeled “A” and “B” for easy tracking of your samples.

4. Insert the EVE™ Cell counting slide with beads into the slide port on the instrument, making sure that the sample side is inserted completely into the instrument.

5. Press **Count beads button**.

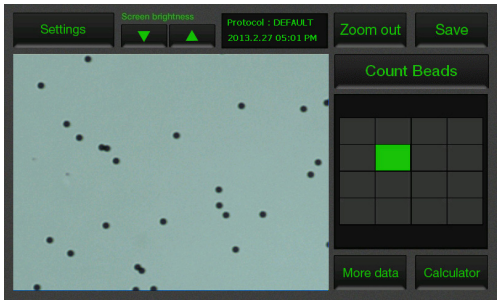


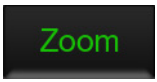
» You can check the result in detail pressing **More data button**.



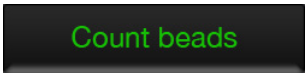
Using the beads mode for counting beads

6. Adjust the bead image by pressing the **Zoom**. Navigate the fields by pressing the location you like to see on the grid.





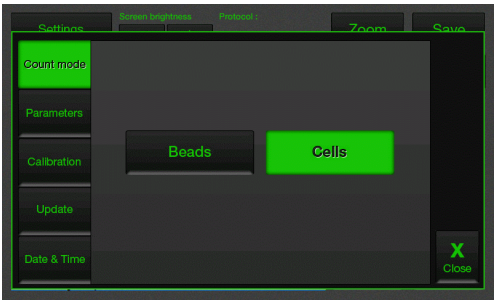
7. When you are satisfied with the image, press the **Count beads.**



8. The instrument takes approximately 20 seconds to count each sample and the bead count is displayed in the screen. Record the bead count.

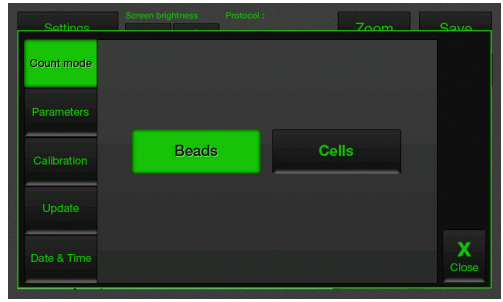
9. To count beads in the other side of the slide chamber (side B), remove the slide after side A is counted, turn the slide around, and reinsert into the slide inlet to repeat the counting procedure.

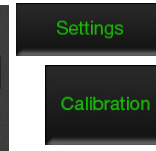
10. After counting beads, place instrument into **Cell Count mode** for counting cells by pressing **Settings** and then pressing the **Cells**.



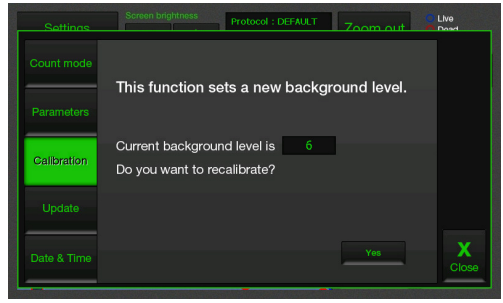
Calibrating

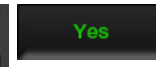
1. Press **Settings** and then press **Calibration**.



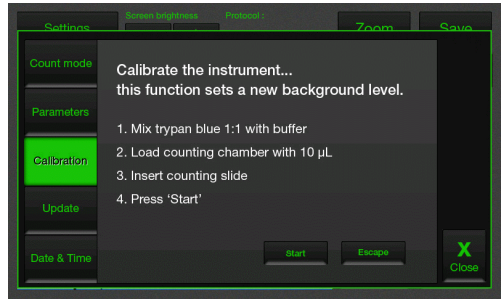


2. Check the current background level and press **Yes** to recalibrate.



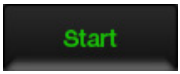


3. To recalibrate the EVE™, mix 10 µL trypan blue solution with 10 µL of a standard buffer, (in a 1:1 ratio) such as phosphate buffered saline (PBS). Mix thoroughly.



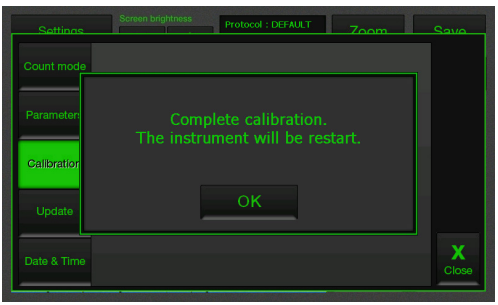
Calibrating

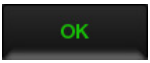
4. Load 10 µL of the sample mixture to the chamber ports on one side of the EVE™ Cell counting slide. Press **Start**.



5. After calibration is completed, press the **OK** to restart the instrument and proceed to cell counting.

There is no need to recalibrate each time the instrument is turned on.





PC software

Installation

The EVE™ is designed for stand-alone use and does not require the use of an external computer.

If you wish to archive data and generate reports, you must transfer data to your computer, and use the EVE™ software to generate and print reports (see instructions, below).

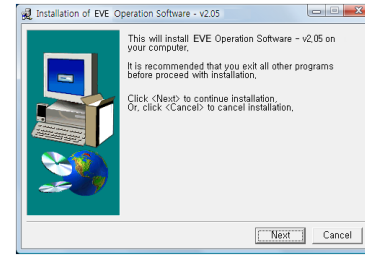
Alternatively, data stored in the .CSV file may be transferred to your computer by the USB drive and imported into any spreadsheet program, without the need for EVE™ PC software.

Computer requirements (EVE™ is not compatible with Macintosh operating systems)

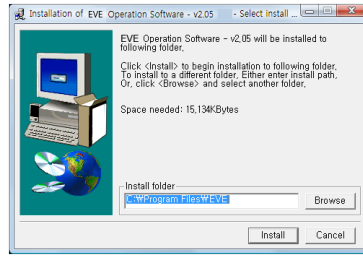
- USB port (1.1 or later, 2.0 is recommended)
- Windows XP /2000/Vista/7

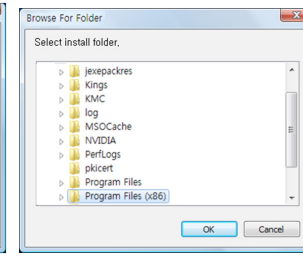
1. Insert the supplied USB drive into the computer. Then open the file "**EVE™ PC Software**".

2. The start-up dialogue of the software will appear. Click '**Next**' to start installation.



3. If you want to change installation folder, click '**Browse**' and choose the location that you want. And click '**Install**'.

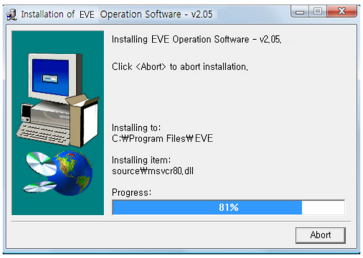




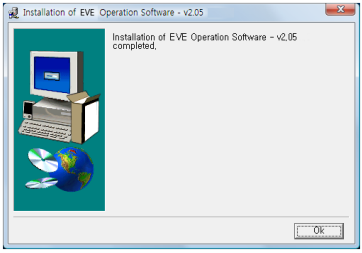
PC software

Installation

4. The computer activates the installation of the Software.

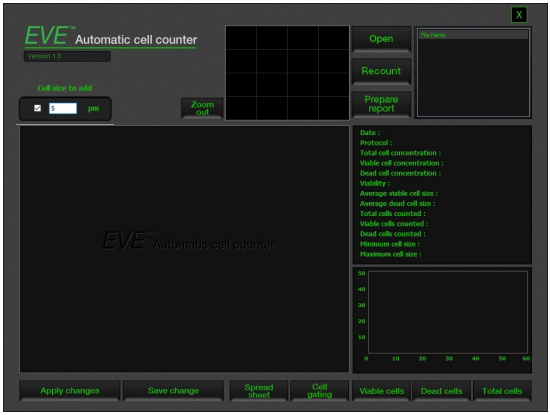


5. Click '**OK**' when Installation is complete.



6. When the software is installed, the PC software icon appears on your desktop.

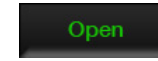
7. Click on the PC software icon on your PC, Start-up screen will be shown.



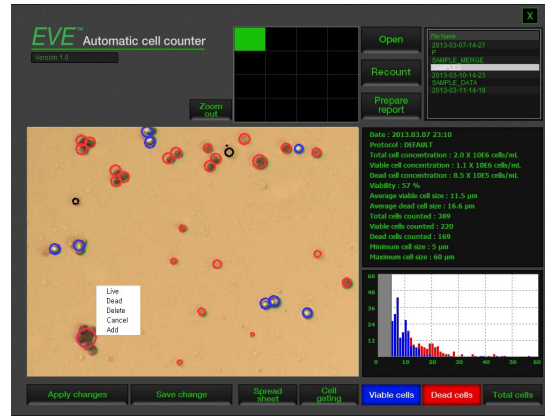
PC software

Open the data

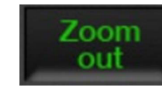
1. Insert the USB to USB port of computer. And Click **Open** to load data files transferred from the instrument.



2. Select the file that need to adjust counting result in different condition.

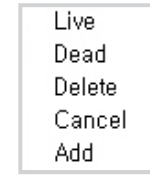


3. Adjust the cell image by pressing the **Zoom**. Navigate the fields by clicking the location you like to see on the grid.



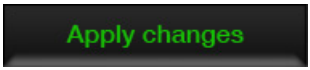
4. If it is necessary, you can adjust cell counting result using **Right** of mouse interface.

You can change the circle mark recognized by counting algorithm of each cell to live or dead. And also, each cell can be deleted or added from counting result.



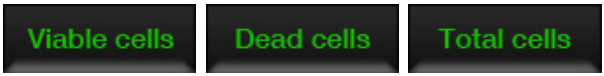
Open the data

5. Click **Apply changes** to make the changes.

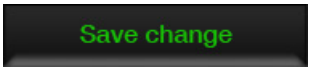


6. You can check cell size graph. Cell size graph will be shown with different color for viable cells, dead cells and total cells.

» Using each button, you can check individual result for viable cells, dead cells and total cells.

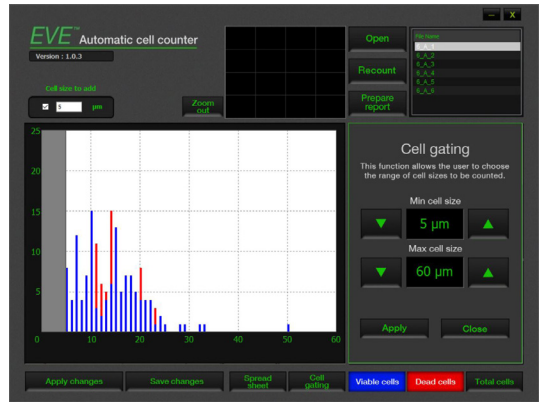


7. If modified image should be saved, then click **Save changes**.



Cell gating

1. Select the saved file you wish to open and adjust range of cell size using **Cell gating**.

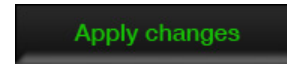


2. Determine the value for minimum cell size and maximum cell size using up and down arrow button.

» **Minimum cell size** is used to determine the low range of cell size to include in the measurement. The algorithm first identifies all objects, and calculates the average size. From the percent of average size setting, the algorithm calculates the smallest object size to include in the final measurement. Adjusting the number up, increases inclusiveness thereby decreasing the lower cell size range.

» **Maximum cell size** is used to determine the high range of cell size to include in the measurement. The algorithm first identifies all objects, then calculates the average size. From the percent of average size setting, the algorithm calculates the largest object size to include in the final measurement.

3. After modifying cell size, click **Apply changes** to make the changes.

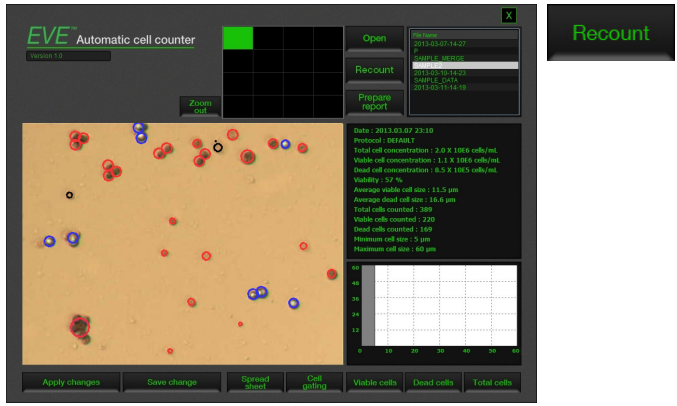


4. You can check modified cell size graph will be shown.

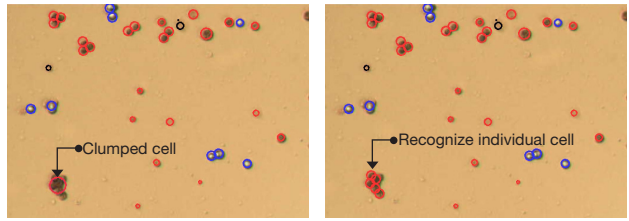
Counting

1. If the **cells clumping** together are existed, these will be counted counted for one cell by counting algorithm. For this reason, the cell counting result may not be accurate.

In this case, select the saved file you wish to open and click Counting button for re-counting the cells, exception algorithm will solve this problem. User can achieve more accurate result.



2. You can check more accurate counting result.



<Result before recounting> <Result after recounting>

» Before recounting, cells clumping together could be counted one cell. But after recounting, software algorithm will recognize and count each of them.

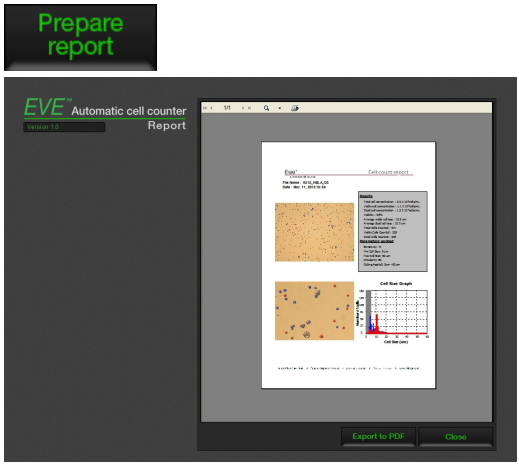
Spread sheet

1. Select the saved file you wish to open and click **Spread sheet**.

2. User can check cell distribution according to cell size differences. And also, this information can be exported by **Export**.

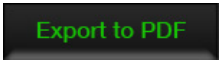
Prepare report

1. Select the saved file you wish to open and click **Prepare report**.

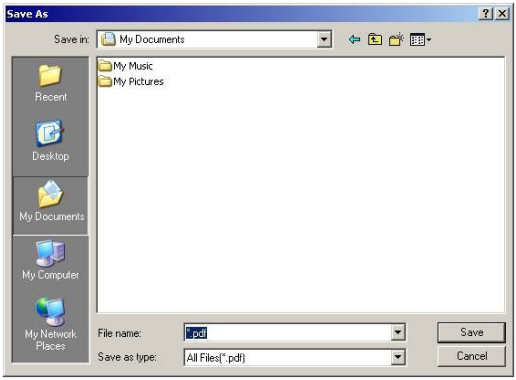


2. You can check preview of report before export to PDF file.

3. Click **Export to PDF** to obtain a printable version of the image and data.



4. Type the file name and select location for saving file.



5. You can check the file on the location you selected.

Cleaning & maintenance

Cleaning and maintenance

Clean the surface of the EVE™ with a damp cloth.
To clean the LCD screen, turn off the EVE™, disconnect the power cable, and clean the LCD screen with a soft cloth lightly moistened with LCD cleansing detergent. Cleaning the screen with excessive force can damage the LCD the screen. Wipe the screen dry immediately. Do not reuse the cell counting slides.

The EVE™ does not need regular maintenance. To troubleshoot problems with EVE™, contact technical support (page 43).
Do not perform any repairs or service on the EVE™ to avoid damaging the instrument.

Troubleshooting

Inaccurate cell count	
Sample handling	• Do not insert the EVE™ Cell counting slide upside-down as this may introduce liquid into the instrument that could damage it. • Do not reuse the EVE™ Cell counting slides, as leftover dye from the previous reading may affect the next reading. • Do not use any other counting slides such as a glass hemocytometer with the EVE™ as it results in inaccurate cell count and may damage the instrument. • Ensure that the sample covers the entire counting area and the EVE™ Cell counting slide is inserted completely into the counter.
Low and high readings	• The EVE™ is designed to read samples from 1×10^4 cells/mL to 1×10^7 cells/mL, with the highest accuracy between 1×10^5 cells/mL and 4×10^6 cells/mL. If your sample is not in this range, you may need to dilute the sample or add more cells and read the sample again.
Poor image quality	• While viewing cells in the Zoom mode, use the focus knob to adjust the image to ensure that live cells have bright centers, and dead cells have dark/blue centers.
Cell clumping	• Ensure the cells are not clumped. · To maintain instrument sensitivity, we recommend that you calibrate the counter each year as described on page 23.

Error code	
	• See page 36 for a description of error codes.

Troubleshooting

Saving and printing problems	
Incorrect USB drive	· Use the USB drive supplied with the counter or an USB 2.0 drive as some types of USB drive are not detected or recorded by the counter. · Do not save too many files in a USB drive as the counter may slow down to read the USB drive.
Accidentally removed the USB drive	· Do not remove the USB drive or turn off the counter when updating. · Do not remove a USB drive when saving or reading data as it may damage the counter.

Instrument not updating firmware	
May be using a corrupted firmware file or a damaged USB drive	· Download a new version of the firmware from the website on a different USB drive and try updating the firmware on the EVE™. Contact technical support (page 43) if the problem persists.

Error codes

This section describes the error codes displayed by the EVE™ when it encounters a problem.
Contact technical support (page 43) for details on error codes and if you need to send the instrument back for servicing.

Position	Reason	Message	Error code
Driver	Camera driver loading fail	**System Error (E0101) Reboot the device or refer to service personnel**	0101
	Camera driver open fail		0102
	Camera driver initializing fail		0103
	GPIO driver loading fail		0104
Preview	Camera preview fail		0201
Memory	Image buffer memory allocation fail		0301
	Camera memory allocation fail		0302

Warranty

Nanoentek warrants that EVE™ will be free from defects in material and workmanship for a period of one (1) year from date of purchase.

If any defects occur in EVE™ during this warranty period,
NanoEntek will repair or replace the defective parts at its discretion without charge.

The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than Nanoentek or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than NanoEntek.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent or sample.

For your protection, EVE™ being returned must be insured against possible damage or loss. NanoEntek cannot be responsible for damage incurred during shipment of a defective instrument. It is recommend that you save the original packing material in which the instrument was shipped. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service,
contact **sales@nanoentek.com** or your local distributor.

Safety precautions

Review and follow the safety instructions below :

· Do not install the instrument in a humid place such as a greenhouse or an incubator to avoid a danger of electric shock. If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to Product Specifications.

· Do not touch the main plug or power cord with wet hands.

· Always ensure that the power supply input voltage matches the voltage available at your location.

· This instrument is air-cooled and its surfaces may become hot during operation. When installing, leave a space of more than 10 cm (4 inches) around the instrument and do not place any objects between the instrument and walls.

· Do not install an instrument on a slant or a place prone to vibrations, which induces the risk of malfunction or damage of the instrument.

· Never insert any objects into the air vents of the instrument as this can result in electric shock, personal injury, and equipment damage.

· Plug the power cord firmly into the wall outlet and AC adapter.

· To avoid potential shock hazard, make sure that the power cord is properly grounded.

· Be sure to position the instrument such that it is easy to disconnect.

· Turn off an instrument before unplugging the power cord and/or moving the instrument.

· If an instrument is dropped or broken, disconnect the power cord and contact a service person The warrant will be void in case of disassembly.

· Use only authorized accessories (adaptor, power cord, and USB driver).

⚠ Warning

Class A equipment is intended for use in an industrial environment. In the documentation for the user, a statement shall be included drawing attention to the fact that there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated **disturbances.**

Consignes de sécurité

Examinez et suivez les consignes de sécurité ci-dessous :

· N'installez pas l'instrument dans un endroit humide comme une serre ou un incubateur pour éviter un risque de choc électrique. Si de l'eau ou tout autre matériau pénètre dans l'instrument, l'adaptateur, ou l'entrées d'alimentation, débranchez le cordon d'alimentation et contactez un technicien de service. Pour l'environnement d'exploitation, reportez-vous aux spécifications du produit.

· Ne touchez pas la fiche ou le cordon d'alimentation principale avec les mains mouillées.

· Assurez-vous toujours que la tension d'entrée d'alimentation correspond à la tension disponible dans votre endroit.

· Cet instrument est refroidi à l'air de sorte que ses surfaces peuvent devenir chaudes pendant le fonctionnement.

Lors de l'installation de l'instrument, laisser un espace de plus de 10 cm (4 pouces) autour de cet instrument et ne placez aucun objet entre l'appareil et le mur.

· N'installez pas l'instrument sur une pente ou un endroit soumis à des vibrations, ce qui induit le risque de dysfonctionnement ou d'endommagement de l'instrument.

· N'insérez jamais aucun objet dans les orifices d'aération de l'instrument, car cela pourrait entraîner un choc électrique, des blessures chez les utilisateurs et des dommages d'équipement.

· Branchez le cordon d'alimentation fermement dans la prise murale et l'adaptateur secteur aussi.

· Pour éviter un risque potentiel de commotion électrique, assurez-vous que le cordon d'alimentation est correctement mis à la terre.

· Assurez-vous de positionner l'instrument de telle sorte qu'il soit facile de débrancher l'instrument.

· Eteignez l'instrument avant de débrancher le cordon d'alimentation et / ou de déplacer l'instrument.

· Si l'instrument est cassé ou qu'il soit tombé, débranchez le cordon d'alimentation et contactez un technicien de service. Ne démontez pas l'instrument et la garantie sera annulée en cas de démontage.

· Utilisez uniquement les accessoires autorisés (l'adaptateur, le cordon d'alimentation, et le lecteur USB).

⚠ Warning

Le produit de classe A est conçu pour l'utilisation dans un environnement industriel. Dans la documentation de l'utilisateur, la déclaration doit être incluse pour attirer l'attention sur le fait qu'il peut y avoir des difficultés potentielles pour assurer la compatibilité électromagnétique dans d'autres environnements, en raison des perturbations rayonnées et conduites par l'électricité.

Safety precautions

Review and follow the safety instructions below :

Symbol	Meaning
	Caution & Warning
	Protective earth (Ground)
	This instrument and consumables conforms to the Declaration of Conformity.
FCC compliance	This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.
	WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE.
	This product conforms to UL61010-1/CSA C22.2 No. 61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements.” Instruments bearing the TUV symbol are certified by TUV Product Services to be in conformance with the applicable safety standards for the US and Canada.

Product specifications

Environmental conditions

Operating power	AC 100 - 240 VAC, 1.5A
Frequency	50 / 60 Hz
Electrical input	12 VDC, 1.0 A
Installation site	Indoor use only
Operating temperature	5 - 40 °C
Maximum relative Humidity	20 - 80 %
Altitude	≤ 2,000 m
Transient category	Installation categories II
Pollution degree	2
Degree of protection	IPX0

EVE™ instrument

Instrument type	Benchtop cell counter
Counting time	< 20 seconds
Cell measurement range (cells/mL)	1×10^4 - 1×10^7
Optimal measurement range (cells/mL)	1×10^5 - 4×10^6
Cell size range	5 - 60 µm
Dimensions	27 cm (W) x 20 cm (D) x 19 cm (H)
Weight	2.1 kg

EVE™ Cell counting slide

Material	Polymethy methacrylate
Dimensions	75 mm (L) x 25 mm (W) x 1.8 mm (H)
Chamber depth	100 µm
Chamber volume	10 µL
EVE™ USB drive	2 GB

Ordering information

The following products can be used with the EVE™ and are available separately from NanoEntek.

Cat. No.	Description	Contents
EVS-050	EVE™ Cell counting slide	50 slides (100 counts, with 1 ea X 1.5 mL of trypan blue (0.4%)
EVS-1000	EVE™ Cell counting slide	1,000 slides (2,000 counts, with 20 ea X 1.5 mL of trypan blue
EVS-5000	EVE™ Cell counting slide	5,000 slides (10,000 counts, with 100 ea X 1.5 mL of trypan blue (0.4%)
EBT-001	Test beads	1mL

Technical support

Visit the our Website at **www.nanoentek.com** for :

· Technical resources, including manuals, FAQs, etc.

· Technical support contact information

· Additional product information and special offers.

For more information or technical assistance, please call or email.

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Website
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EVE™

Automated cell counter

NESMU-EVE-001E (V.1.6)

NanoEntek

NanoEntek, Inc.
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NESMU-CS-001E (V.0.2)

CE

C-Slide

Instruction

Cell counting chamber slides

CS-050

NanoEntek

NanoEntek
Website : www.nanoentek.com
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The information in this manual is described as correctly as possible but may be changed without prior consent or notification.

Revision history : **V.0.2 Date: JUN 2025**

Safety Precautions

For analyzing hazardous or potential infectious materials:

- Take necessary precautions
- Handle with care
- Dispose in an appropriate way

Long exposure to solvents will cause the slide to warp.
Xylene and toluene based mounting media should be avoided.
Glycerol, gelatin, and other aqueous-based media are recommended.

Safety Symbols

The safety symbols on the C-Slide are intended to inform you of potential danger or a particular caution. Before use, please read and the consult the guide for the symbols and their meanings.

REF Catalogue number/Reference number

LOT Batch code (Lot Number)  Temperature limitation

 Do not reuse  Use by CE marking

 Consult instructions for use  Manufacturer

US Corporation US Corporation European Corporation European Corporation

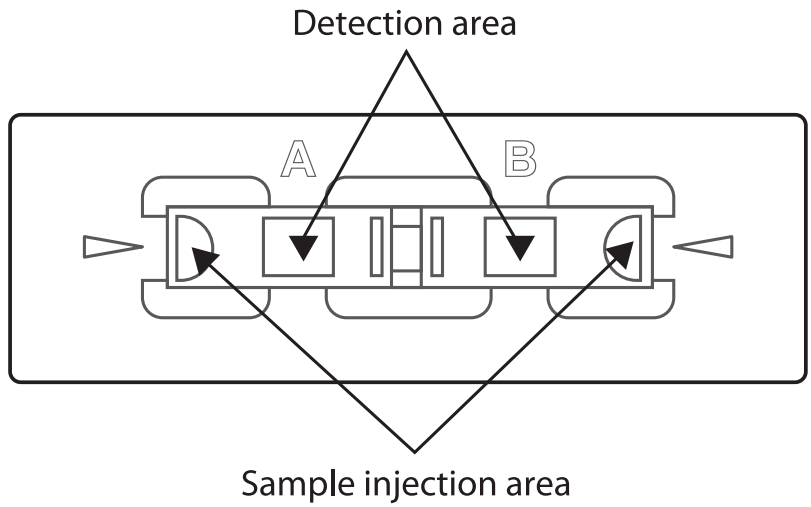
NOTE : The C-Slide is for **single use** only. **Do not reuse.**
C-Slide should be used immediately after unsealing.

Introduction

C-Slide is a cell counting chamber slide used with automated cell counters.

It consists of two separate enclosed chambers each with two ports for sample injection. Cell counting occurs in a central location of the counting chamber. It can be used to count one sample in duplicate or to count two different samples.

C-Slide eliminates need to slide or coverslip washing thereby reducing the exposure to potentially infectious sample and contamination.



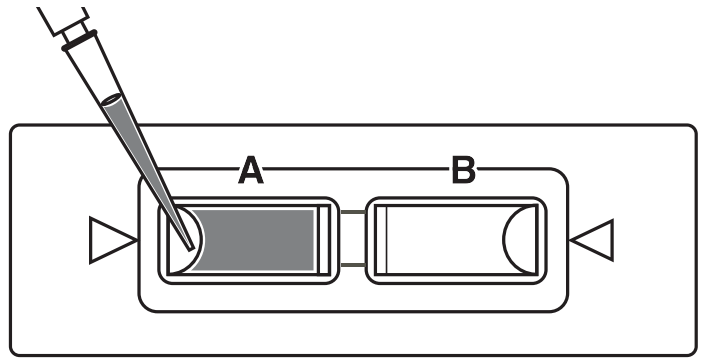
Procedure

Instructions for using C-Slide, cell counting chamber slides, for cell counting with trypan blue stain are described below.

[C-Slide]

1. Load Sample

- Mix together 10 µL of sample and 10 µL of 0.4% trypan blue stain.
- Load 10 µL of the sample mixture into the half moon shaped chamber port (marked A or B) on C-Slide. You may load one chamber or both chambers.



[Automated cell counter]
*** For more instructions, please refer to the manual of each instrument.**

2. Insert Slide

- Insert slide into the slide port of the instrument, sample side first.

3. Optimize Image

- Adjust focus of the image according to instrument you are using.

4. Count Cells

- Count cells with a function of your automated instrument.
- Check results on the screen.
- To read the second chamber, remove and turn around the slide. Then repeat the procedures with the another slide.

Trouble shooting

In case of poor visibility results,

- Carefully load samples into the slide in order to prevent the introduction of air bubbles.
- Observe after removing the dust from samples before a test.
- Do not rub or touch the detection area of slide.
- Adjust the focus of the automated cell counter.
- Test again with a clean sample.

Storage and Handling

All unopened/opened materials are stable until the expiration date on the label when stored at the room temperature. Reagent stability has been demonstrated for 24 months from the date of manufacture. The expiration date is indicated on the front side of outer box.

CE

EVE™ PLUS

Automated cell counter

User Manual

NanoEntek

EVE™ PLUS User Manual
Website: www.nanoentek.com
E-mail: sales@nanoentek.com

Manufactured by

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The information contained in this document is subject to change without notice.
Firmware and software changes and updates may change without prior consent or notification.

Documentation: **NESMU-EVE2-001E**

Revision history: V.0.0 JUN 2019
V.1.0 NOV 2019
V.1.5 MAR 2020
V.2.0 AUG 2020
V.2.1 FEB 2022
V.2.5 OCT 2024

Table of contents

Product contents

EVE™ PLUS is shipped with the following components.
Please check that all items listed below were shipped, receiving the instrument. If any items are missing or damaged, contact your local distributor or e-mail sales@nanoentek.com.

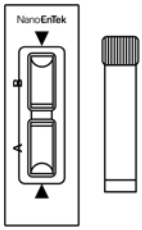
Automated cell counter

1 EA

Cat. No. EVE-MC2

Cell counting slides
with 1.5 mL of Trypan blue (0.4%)

1 BOX (50 slides/box)



Cat. No. EVS-050

Power cord
Europe/Korea, USA/Japan,
Australia, UK, or China

1 SET

Adaptor

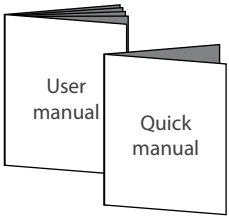
1 SET

Wifi dongle

1 EA

User manual & Quick manual

1 EA



Product overview

EVE™ PLUS Automated cell counter uses state-of-the-art optics and image analysis for automatic cell counting. EVE™ PLUS is a benchtop counter designed to measure cell count and viability (live, dead, and total cells) accurately and precisely, sing the standard trypan blue and erythrosin B solution.

Using the same amount of sample that you currently use with the hemocytometer, EVE™ PLUS takes less than 1 second per sample for a typical cell count with manual focus option and is compatible with a wide variety of eukaryotic cells and provides information on cell size.

EVE™ PLUS is supplied with disposable EVE™ Cell counting slides that contain two enclosed chambers to hold the sample to allow you to measure two different samples or perform replicates of the same sample. The cell counting occurs in the central location on the counting slide and the volume counted is 0.4 µL, the same as counting four (1 mm × 1 mm) squares in a standard hemocytometer.

*NOTE: Use of Erythrosin B solution and the method mode is available with the upgraded EVE Plus.

Features and benefits

• User-friendly, compact design for simple, fast, automated cell count and viability measurements within 1 second.

• It provides data on cell size and is compatible with various types of eukaryotic cells, regardless of whether the cell size is small or large.

• Measures cell concentrations ranging from 1×10^4 to 2×10^7 cells/mL and cells with sizes ranging from 5 µm to 60 µm.

• Provides the clumpy cell counting function to get more accurate results.

• Uses disposable cell counting slides that you can eliminate washing steps and cross contamination between samples with.

• Up to 500 test results are automatically saved in the DATA tab.

• Presents comprehensive data with graphical reports and as a .CSV (comma separated value) file for sample comparisons.

Front view

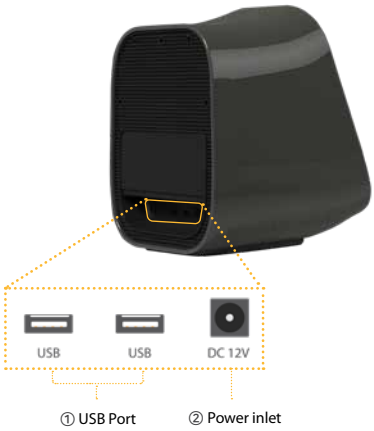
Item	Description
① LCD touch screen	Located in the front of the instrument contains buttons for all the functions needed and displays data from the cell count.
② Slide slot	The slide slot is used to insert the EVE™ Cell counting slide containing the sample with trypan blue or erythrosin B solution into the counter for analysis.

Side view

Item	Description
③ Image adjustment (focus) knob	The image adjustment (focus) knob is used to adjust the image quality to obtain better contrast between live (bright centers) and dead (dark blue centers) cells by manually. This is important to obtain accurate cell counts and viability measurements.
④ USB port	The USB port allows you to transfer and save the cell count data and image to your computer for record keeping and printing purposes. Any other standard USB drive can be used for data transfer.
⑤ Power button	To turn the power on and off, press and hold for 3 seconds. The unlighted status indicates that the instrument is off; the red status light indicates that the instrument is on.

Rear view

Control buttons	Description
① **USB port**	The USB port allows you to transfer and save the cell count data and image to your computer for record keeping and printing purposes. Any standard USB drive can be inserted into the USB port for data transfer.
② **Power inlet**	Connect the counter to an electrical outlet using the supplied power cord and the appropriate plug, based on the electrical outlet configuration in your country.



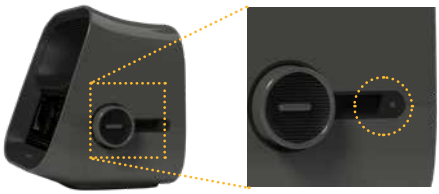
Install EVE™ PLUS

1. After unpacking the instrument, place the instrument on a flat and dry surface.

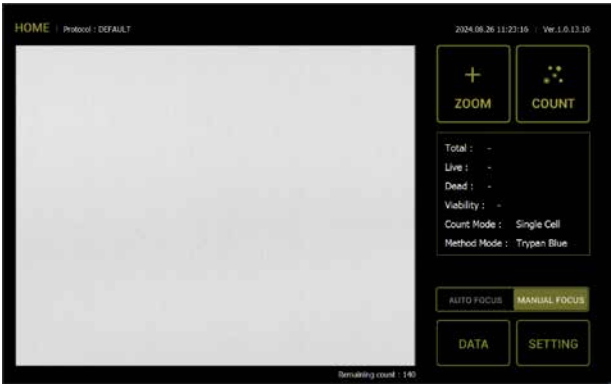
2. Connect adaptor and power cord, then plug the power cord to EVE™ PLUS.

3. Plug the power cord into the electrical outlet. Be sure to use only the power cord supplied with your instrument. Powering the instrument with an unapproved power cord may damage the instrument.

4. When you are ready to use, start the EVE™ PLUS by pressing and hold 3 seconds the Power button.



5. When the instrument is turned on, the startup screen is displayed. Here you can proceed immediately to cell counting, set up the instrument for cell types, or adjust the parameter for cell counting.



Activation code

To use the EVE™ PLUS for the first time, you need the activation code, which is in the EVE™ slide box enclosed with the instrument. Entering the provided code into the pop up window allows normal cell counting.

This code has a limit on the number of uses, and it is possible to use the counting function of 1000 times per code.

If the limit is exceeded, this popup window will reappear.
In this case, you must enter a new code from the new box.

* This code is intended to provide accurate calculation results using correct consumables.

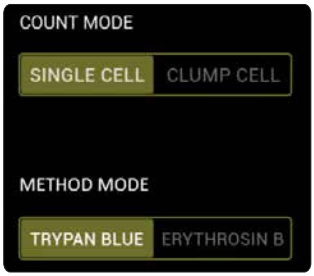
Setting menu

Press SETTING from the startup screen to display settings.
The setting menu allows you to set up the following:

- MODE to operate the instrument for cell count method (choose SINGLE CELL or CLUMP CELL in count mode) or solution (choose TRYPAN BLUE or ERYTHROSIN B in method mode).
- PARAMETER (see below and next page for details)
- CALIBRATION to calibrate the instrument image background level (page 12)
- UPDATE to install new firmware versions as they become available
- DATE to set up date and time (page 14)
- WIFI to connect to the internet to send results via mail

Mode

Select count mode and method mode for cell counting. Please choose counting mode (SINGLE CELL or CLUMP CELL) and method mode (TRYPAN BLUE or ERYTHROSIN B).
SINGLE CELL refers to cells that exist individually, and counting is performed using the SINGLE CELL MODE.



CLUMP CELL refers to cells that do not exist individually but instead form clusters or aggregates. To ensure accurate counting, they must be imaged using the CLUMP MODE.

Parameter

1. Press PARAMETER from the setting screen to display parameters screen.

Parameter function allows you to change the image analysis algorithm for specific or mixed cell types, and the specific parameters must be determined empirically.

*NOTE: Use of Erythrosin B solution and the method mode is available with the upgraded EVE Plus.

Parameter

2. The parameters are described below:

[Sensitivity]
Sensitivity (refers to the contrast of the objects against the background). Adjusting the sensitivity higher makes instrument more sensitive to objects; useful for cells that do not stain well with trypan blue while adjusting the sensitivity lower makes the instrument less sensitive and is useful if there is a lot of background.

[Minimum cell size]
Minimum cell size is used to determine the low range of cell size to include in the measurement. The algorithm first identifies all objects, and calculates the average size (e.g., 15 µm). From the percent of average size setting, the algorithm calculates the smallest object size to include in the final measurement (e.g., 70% of 15 is 10.5 µm; 15–10.5 = 4.5 µm; 4.5 µm) would be the smallest particle included in the count. Adjusting the number up, increases inclusiveness thereby decreasing the lower cell size range (e.g., 50% of 15 is 7.5 µm; 15–7.5 = 7.5 µm).

[Maximum cell size]
Maximum cell size is used to determine the high range of cell size to include in the measurement. The algorithm first identifies all objects, then calculates the average size (e.g., 15 µm). From the percent of average size setting, the algorithm calculates the largest object size to include in the final measurement (e.g., 200%; 200% of 15 µm = 30 µm; 30 µm) is the largest cell size included in the measurement.

Circularity is used to determine the objects to include in the measurement based on roundness. Increasing the value from 80% requires objects to be more round for inclusion in the measurement. Decreasing the value from 80% allows objects to be less round. Adjusting this may be useful if the cell type is not particularly circular or perhaps oddly shaped.

[Maximum cell size]
After modifying any parameters,
press APPLY button to make the changes.

To restore default parameters, press DEFAULT button.

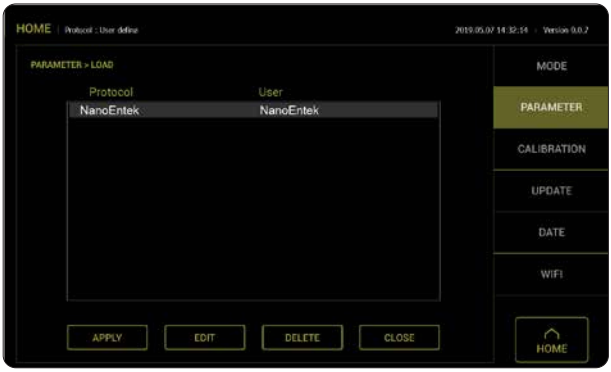
3. Press NEW button to create a new protocol.

Parameter

4. Type protocol name and user name in the appropriate fields, and press SAVE button again.

5. Once a protocol is saved, it is available for use at any time. Press LOAD button.

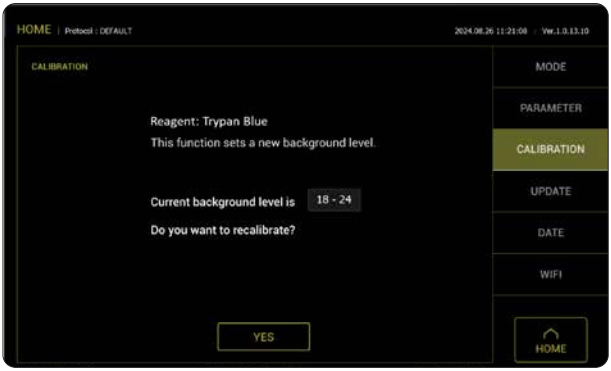
6. The protocol appears in the protocol menu.
Use the up and down arrows to find your saved protocol.
To use the protocol, select one of protocol and press APPLY button.



» Press DELETE button to delete protocol.
Press EDIT button to edit protocol.
Press CLOSE button to exit the screen.

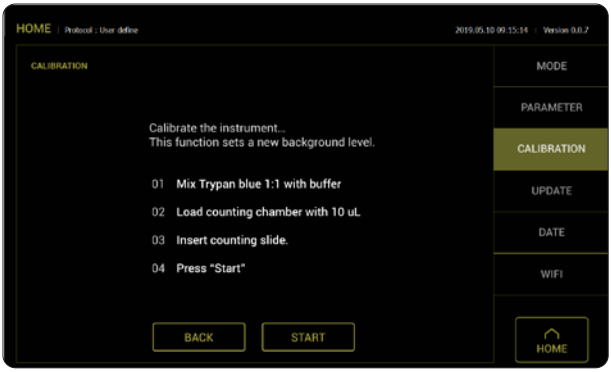
Calibration

1. Press SETTING and then press CALIBRATION button.



2. Check the current background level and press YES button to recalibrate.

3. To recalibrate the EVE™ PLUS, mix 10 µL trypan blue or 10 µL erythrosin B solution with 10 µL of a standard buffer, (in a 1:1 ratio) such as cell culture media. Mix thoroughly.
Before recalibration, match the solution and METHOD MODE in MODE menu.

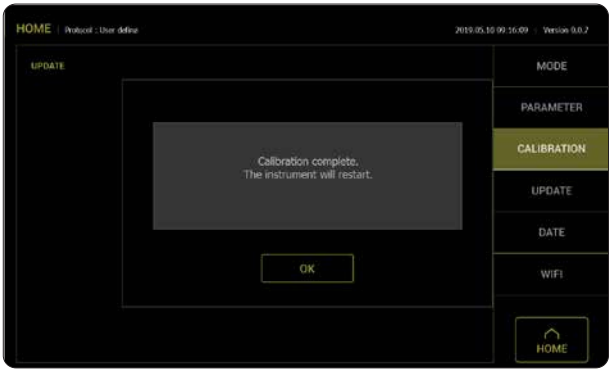


Calibration

4. Load 10 µL of the sample mixture onto the EVE cell counting slide and insert in into the slide slot. Press START button.

5. After calibration is completed, press the OK button to restart the instrument and proceed to cell counting.

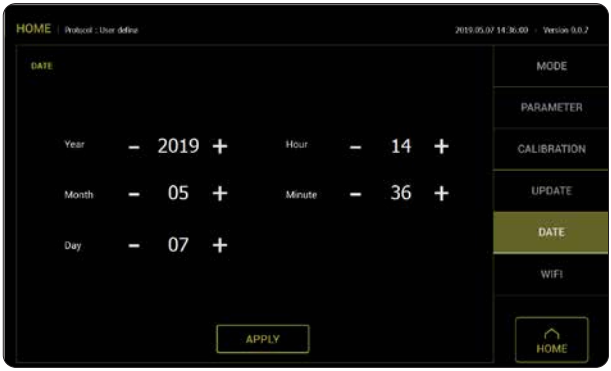
There is no need to recalibrate each time the instrument is turned on.



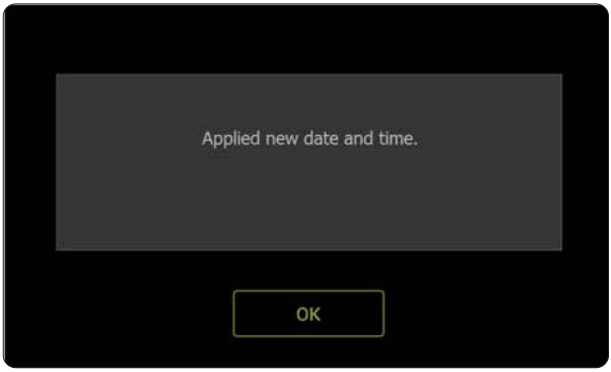
Date

1. Press SETTING button and then press DATE button.

2. The Date/Time properties screen is displayed. To select the year, month, day, hour and minute, using the plus and minus button select the number.



3. Press APPLY button to make the Date/Time changes.



4. Press OK button to exit the screen.

The updated Date/Time is displayed on the top of the window.
Once the date/time is set, there is no need to set it each time the instrument is turned on.

Recommend actions

To obtain the best results, follow these recommendations:

1. Wear protective gloves during sample handling.

2. Do not touch the optical surfaces of the EVE™ Cell counting slides. Hold the cell counting slides by the edges.

3. Use the EVE™ PLUS at room temperature only (5 - 40 °C).

4. For accurate viability count results, ensure the counting area is covered with cell suspension and count cells within 3 minutes of mixing the cells with trypan blue solution as trypan blue is toxic to cells. Alternatively, Use the Erythrosin B solution that less toxic to the cell.

5. For best data with biological samples, we recommend counting at least two samples and taking an average.

6. The calibration completed EVE PLUS will be supplied. If necessary, re-calibrate your instrument. And recommend the recalibration when change the METHOD MODE (page 12).

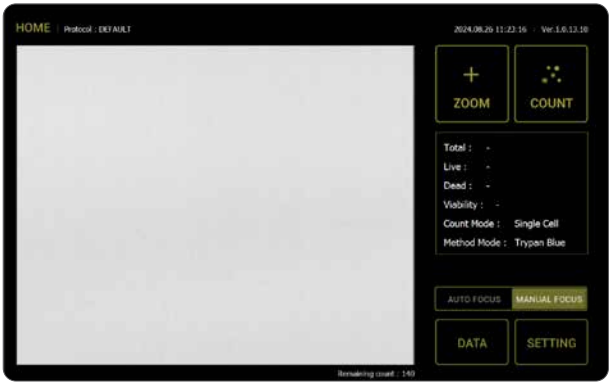
7. The EVE™ PLUS storage holds up to 500 data. If need a test report, save the data to the USB drive whenever you want. You may transfer the data to your PC, using the USB drive as described in transferring data to a PC (page 29).

8. After using EVE™ PLUS, appropriately dispose slides as biohazardous waste.

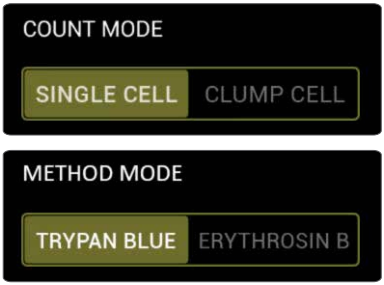
9. For accurate viability count results, do not reuse the cell counting slides. And when the sample injected onto the slide start to dry, do not use it.

Cell counting (Auto focus)

1. Press the power button to start the instrument.
The Start-up screen is displayed.

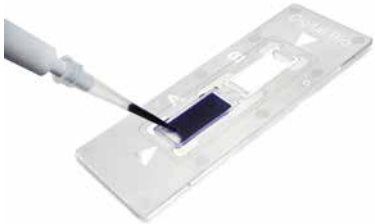


2. EVE™ PLUS is set to the COUNT MODE and METHOD MODE used previously. If you want to change the COUNT MODE and METHOD MODE, use the MODE option in the SETTING.



3. Mix well the 10 µL of your sample and the 10 µL of 0.4% trypan blue or 0.05% erythrosin B solution. (in a 1:1 ratio)

4. Load 10 µL of the sample mixture on EVE™ Cell counting slide (side A) using a pipette.
The chambers on the slide are labeled "A" and "B" for easy tracking of your samples.

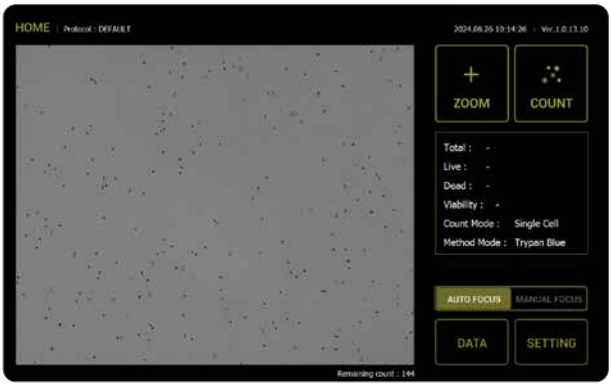


*NOTE: Use of Erythrosin B solution and the method mode is available with the upgraded EVE Plus.

Cell counting (Auto focus)

5. Insert the EVE™ Cell counting slide, sample side (side A) first into the slide slot until you hear a soft click. Each chamber is counted separately.

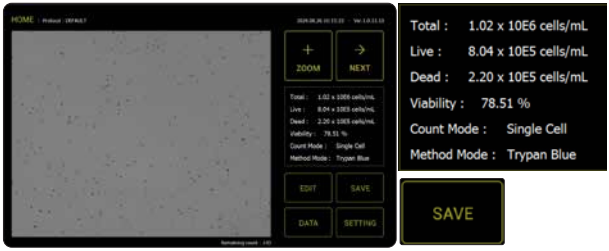
6. Before cell counting, press the AUTO FOCUS button.



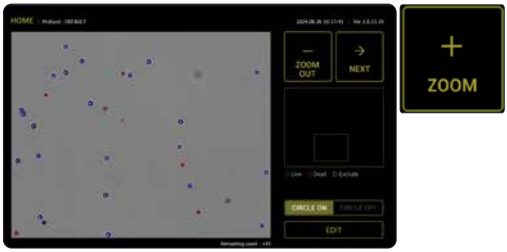
7. Press the COUNT button.

Cell counting (Auto focus)

8. Total cell counting and viability result are shown as below in 10 seconds. To save the data, insert a USB drive and press SAVE button (see page 29).



9. To see more details counting results on the screen, press the ZOOM button. Then, use the navigator can see detail count result and viability result with blue and red color circle.

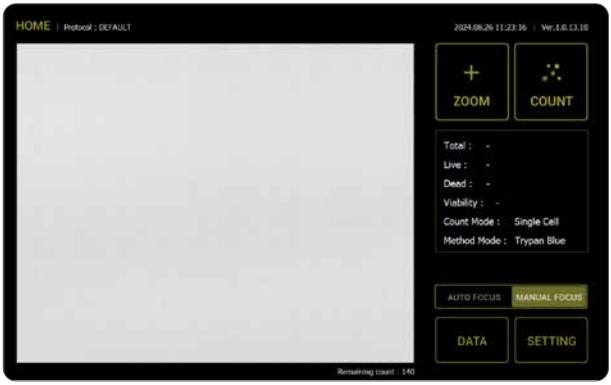


10. To see more details on the data as well as graphical representation of the data, press the EDIT button (see page 28).

» Press the HOME button to return to the main screen.

Cell counting (Manual focus)

1. Pressing and hold 3 seconds the Power button to start the instrument. The Start-up screen is displayed.



2. Mix well the 10 µL of your sample and the 10 µL of 0.4% trypan blue or 0.05% erythrosin B solution. (in a 1:1 ratio)

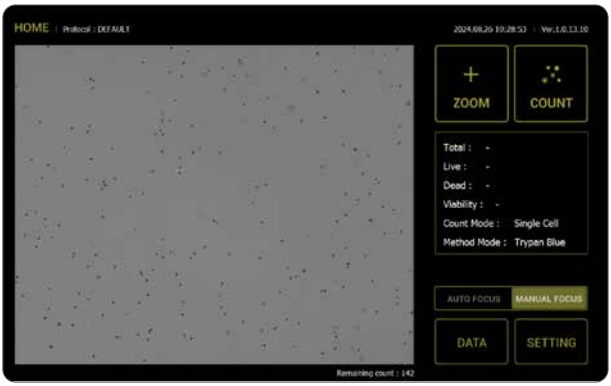
3. Load 10 µL of the sample mixture on EVE™ Cell counting slide (side A) using a pipette.
The chambers on the slide are labeled "A" and "B" for easy tracking of your samples.

4. Insert the EVE™ Cell counting slide, sample side (side A) first into the slide slot until you hear a soft click. Each chamber is counted separately.

*NOTE: Use of Erythrosin B solution and the method mode is available with the upgraded EVE Plus.

Cell counting (Manual focus)

5. Before cell counting, press the MANUAL FOCUS button.

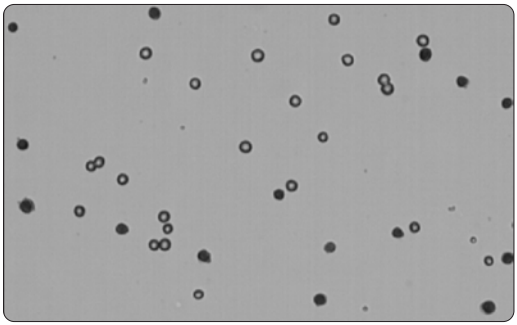


6. For the correct focusing by pressing the ZOOM button. Select by pressing the location you like to see on the navigator. While viewing cells in the zoom mode, use the focus knob to adjust the image.

For the manual focus guide, see the following below image.

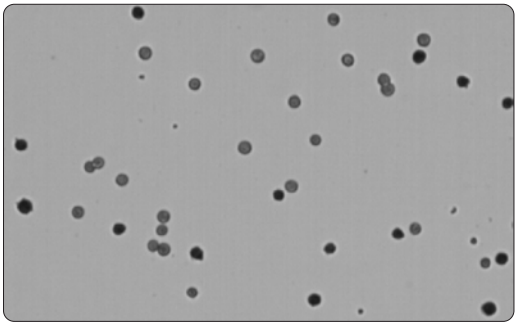
Cell counting (Manual focus)

Optimize the image for analysis such that:



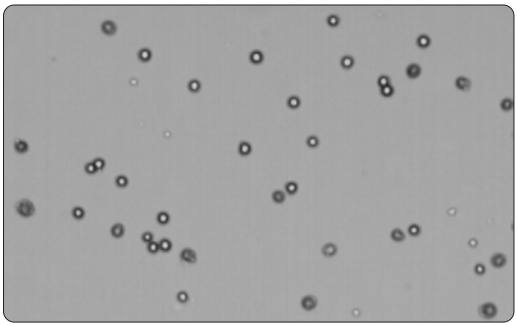
<Correct Image>

Live cells have bright centers and dark edges.
Dead cells have a uniform blue color throughout the cell with no bright centers.



<Incorrect Image 1>

Live cells have dark centers and are counted as the dead cells.



<Incorrect Image 2>

Dead cells have bright centers are counted as the live cells.

Cell counting (Manual focus)

7. When you are satisfied with the image, press COUNT button.

8. With in 1 second to count each sample, and the cell count for live, dead, and total cells as well as percentage viability is displayed on the screen. To save the data, insert a USB drive and press the SAVE button (see page 29).

9. To see more details on the data as well as graphical representation of the data, press the EDIT button (see page 28).

» Press the HOME button to return to the main screen.

Cell counting (Manual focus)

10. The CALCULATOR button allows you to quickly calculate adjustments to the cell suspension to obtain a desired concentration.

11. To count the cells in the other side of the slide (side B), remove the EVE™ slide after side A is counted by pushing in the slide slightly and then pulling the slide out. Turn the slide around and reinsert into the slide inlet and repeat the procedure.

12. EVE™ PLUS storage holds up to 500 data. If need a test report, save the data to the USB drive whenever you want. You may transfer the data to your PC, using the USB drive as described in transferring data to a PC (page 29).

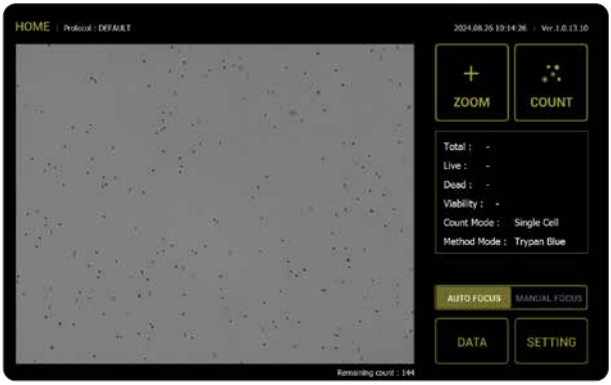
13. After recording or saving the data, remove and discard the slide appropriately as bio-hazardous waste.

14. At this point, the EVE™ PLUS is ready for another sample. If you are not using the instrument, press the power button to turn off the instrument.

» If the touch screen is not responding, you can turn off the instrument by pressing and holding the Power button for 3 seconds.

How to use Test beads

1. Press the AUTO FOCUS button.



2. Press SETTING and then select SINGLE CELL on COUNT MODE and TRYPAN BLUE on METHOD MODE.
(if use the erythrosin B instead of trypan blue, select the ERYTHROSIN B on METHOD MODE.)

3. Put 10 µL of beads to 10 µL of 0.4 trypan blue or 10 µL 0.05% erythrosin B solution, and mix well. Mix thoroughly by pipetting up and down.

4. Load 10µL of sample mixture on EVE™ Cell counting slide (side A or B) using pipette. The two chambers of the slide are labeled "A" and "B" for easy tracking of your samples.

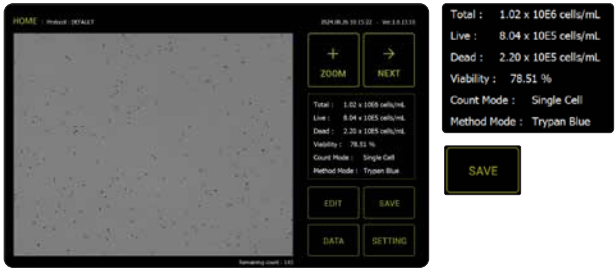
5. Insert the EVE™ Cell counting slide with beads into the slide port on the instrument, making sure that the sample side is inserted completely into the instrument.

6. Press COUNT button.

*NOTE: Use of Erythrosin B solution and the method mode is available with the upgraded EVE Plus.

How to use Test beads

7. The results will be displayed on the screen.



8. Check if the given range of Test beads and counted Dead cells are in the same range.

* Given range of Test beads : approx. $9.60x10^5$ – $1.44x10^6$ beads/mL
* Due to debris, live cells may be displayed

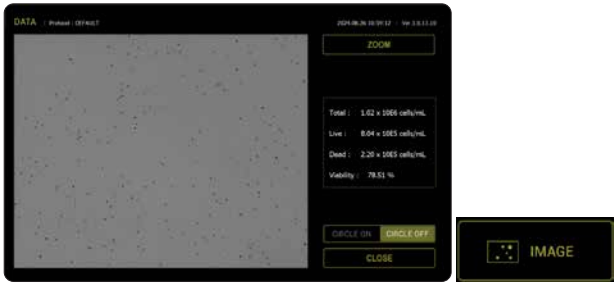
9. To count beads in the other side of the slide chamber (side B), remove the slide after side A is counted, turn the slide around, and reinsert into the slide inlet to repeat the counting procedure.

Check the image

1. Push the DATA button to checking data lists.

2. Data lists and properties screen is displayed.
When cell counting is done, all the information is automatically stored in the DATA and up to 500 lists can be stored.

3. After Choose result on the lists, press IMAGE button to check detail.



Check the image

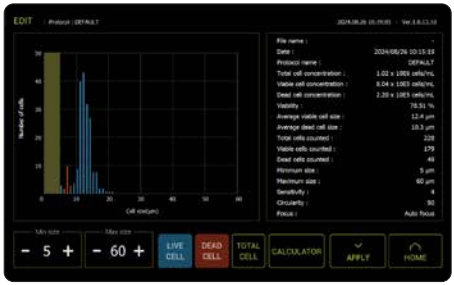
4. To see more details counting results on the screen, press the ZOOM button.

5. Choose CIRCLE ON button to see detail counting result. Then, use the navigator can see detail count result and viability result with blue and red color circle.

6. Press CLOSE button to exit the screen.

Edit the histogram

1. Choose result on the lists, then press HISTOGRAM button to check detail.



2. Determine the value for minimum cell size and maximum cell size using up and down arrow button.

» Minimum cell size is used to determine the low range of cell size to include in the measurement. The algorithm first identifies all objects, and calculates the average size. From the percent of average size setting, the algorithm calculates the smallest object size to include in the final measurement. Adjusting the number up, increases inclusiveness thereby decreasing the lower cell size range.

» Maximum cell size is used to determine the high range of cell size to include in the measurement. The algorithm first identifies all objects, then calculates the average size. From the percent of average size setting, the algorithm calculates the largest object size to include in the final measurement.

3. After modifying cell size, click APPLY button to make the changes.

4. Press BACK button to exit the screen.

5. You can check modified cell size graph and result will be apply on data lists.

Data export (Report & image)

1. To archive your data or generate a printed report, insert USB drive into the USB port.

2. Save your data on the USB drive by pressing the SAVE button on the main screen and data menu.

2-1) SAVE button on the main screen.
In case of save to USB immediately after cell counting.

2-2) SAVE button on the DATA tap.
In case of save to USB through DATA tap lists.

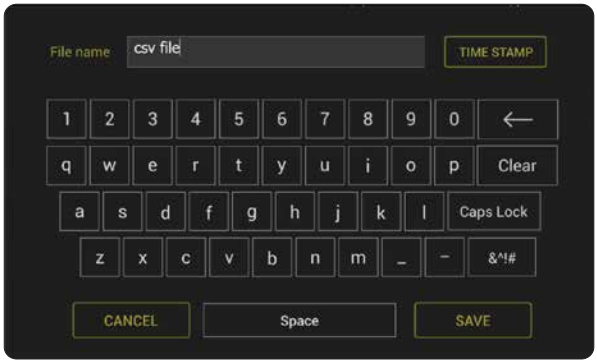
3. Enter the file name using the keypad buttons displayed.

4. After press SAVE button, the image and the data report are saved in USB drive.

DATA export (CSV file)

1. Save CSV file on the USB drive by pressing the SAVE DATA(.CSV) button on the main screen and data menu. The numerical data is also automatically saved as a .CSV file that can be opened with any spreadsheet program.

2. Enter the file name using the keypad buttons displayed on the save menu.

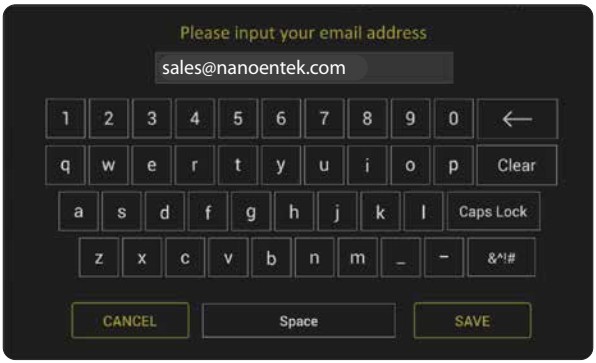


3. Transfer the USB drive to the USB port on your PC. You may open the .CSV file using a spreadsheet program.

DATA export (Mail)

1. Push the MAIL button to transfer data.

2. Enter the file name using the keypad buttons displayed on the save menu.



* To send data by e-mail, it is necessary to connect Wi-Fi.
It can be set on Wi-Fi option in the SETTING menu.

Maintenance and cleaning

Clean the surface of the EVE™ PLUS with a damp cloth.
To clean the LCD screen, turn off the EVE™ PLUS, disconnect the power cord, and clean the LCD screen with a soft cloth lightly moistened with LCD cleansing detergent. Cleaning the screen with excessive force can damage the LCD the screen. Wipe the screen dry immediately. Do not reuse the cell counting slides.

The EVE™ PLUS does not need regular maintenance. To troubleshoot problems with EVE™ PLUS, contact technical support (page 41).
Do not perform any repairs or service on the EVE™ PLUS to avoid damaging the instrument.

Trouble shooting

Inaccurate cell count

Problem	Solution
Sample handling	• Do not insert the EVE™ Cell counting slide upside-down as this may introduce liquid into the instrument that could damage it. • Do not reuse the EVE™ Cell counting slides, as leftover dye from the previous reading may affect the next reading. • Do not use any other counting slides such as a glass hemocytometer with the EVE™ PLUS as it results in inaccurate cell count and may damage the instrument. • Ensure that the sample covers the entire counting area and the EVE™ Cell counting slide is inserted completely into the counter.
Low and high readings	• The EVE™ PLUS is designed to read samples from 1×10^4 cells/ mL to 2×10^7 cells/mL, with the highest accuracy between 1×10^5 cells/mL and 4×10^6 cells/mL. • If your sample is not in this range, you may need to dilute the sample or add more cells and read the sample again.
Poor image quality	• While viewing cells in the zoom mode, use the focus knob to adjust the image to ensure that live cells have bright centers, and dead cells have dark/blue centers.
Clumpy cell	• Ensure the cells are not clumped. • To maintain instrument sensitivity, we recommend that you calibrate the counter each year as described on page12.

Trouble shooting

Saving and printing problems

Problem	Solution
Incorrect USB drive	• Use the USB drive supplied with the counter or an USB 2.0 drive as some types of USB drive are not detected or recorded by the counter. • Do not save too many files in a USB drive as the counter may slow down to read the USB drive.
Accidentally removed the USB drive	• Do not remove the USB drive or turn off the counter when updating. • Do not remove a USB drive when saving or reading data as it may damage the counter.

Instrument not updating software

Problem	Solution
May be using a corrupted software file or a damaged USB drive	• Download a new version of the software on a different USB drive and try updating the software on the EVE™ PLUS. Contact technical support (page 41) if the problem persists.

Warranty

NanoEntek warrants that EVE™ PLUS will be free from defects in material and workmanship for a period of one (1) year from date of purchase.

If any defects occur in EVE™ PLUS during this warranty period, NanoEntek will repair or replace the defective parts at its discretion without charge.

The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than NanoEntek or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than NanoEntek.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent or sample.

For your protection, EVE™ PLUS being returned must be insured against possible damage or loss. NanoEntek cannot be responsible for damage incurred during shipment of a defective instrument. It is recommend that you save the original packing material in which the instrument was shipped. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service,
Contact **sales@nanoentek.com** or your local distributor.

Product specifications

Environmental conditions	
Operating power	100 - 240V~, 1.5A
Frequency	50 / 60 Hz
Electrical input	12 VDC, 3.0 A
Installation site	Indoor use only
Operating temperature	5 - 40 °C
Maximum relative humidity	20 - 80 %
Altitude	≤ 2,000 m
Transient category	Installation categories II
Pollution degree	2
Degree of protection	IPX0

EVE™ PLUS instrument

Instrument type	Benchtop cell counter
Counting time	< 1 second (manual focus) < 10 seconds (auto focus)
Cell measurement range (cells/mL)	$1 \times 10^4 – 2 \times 10^7$
Optimal measurement range (cells/mL)	$1 \times 10^5 – 4 \times 10^6$
Cell size range	5 - 60 µm
Dimensions	274 (W) x 333 (H) x 274 (L) mm
Weight	4 kg

EVE™ Cell counting slide

Material	Polymethy methacrylate
Dimensions	25 (W) x 1.8 (H) x 75 (L)mm
Chamber depth	100 µm
Loading volume	10 µL

Ordering information

The following products can be used with the EVE™ PLUS and are available separately from NanoEntek.

Cat. No.	Description	Contents
EVS-050	**EVE™ Cell counting slide**	**50 slides** 100 counts, with 1 ea X 1.5 mL of trypan blue (0.4%)
EVS-1000	**EVE™ Cell counting slide**	**1,000 slides** 2,000 counts, with 20 ea X 1.5 mL of trypan blue (0.4%)
EVS-5000	**EVE™ Cell counting slide**	**5,000 slides** 10,000 counts, with 100 ea X 1.5 mL of trypan blue (0.4%)
EBB-001	**Test beads** **Concentration(avg.) 1.0 X 10^6**	**1 mL**

Safety precautions

Review and follow the safety instructions below:

• Do not install the instrument in a humid place such as a greenhouse or an incubator to avoid a danger of electric shock. If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to Product Specifications.

• Do not touch the main plug or power cord with wet hands.

• Always ensure that the power supply input voltage matches the voltage available at your location.

• This instrument is air-cooled and its surfaces may become hot during operation. When installing, leave a space of more than 10 cm (4 inches) around the instrument and do not place any objects between the instrument and walls.

• Do not install an instrument on a slant or a place prone to vibrations, which induces the risk of malfunction or damage of the instrument.

• Never insert any objects into the air vents of the instrument as this can result in electric shock, personal injury, and equipment damage.

• Plug the power cord firmly into the wall outlet and AC adapter.

• To avoid potential shock hazard, make sure that the power cord is properly grounded.

• Be sure to position the instrument such that it is easy to disconnect.

• Turn off an instrument before unplugging the power cord and/or moving the instrument.

• If an instrument is dropped or broken, disconnect the power cord and contact a service person The warrant will be void in case of disassembly.

• Use only authorized accessories (adaptor, power cord, and USB drive).

WARNING

Class A equipment is intended for use in an industrial environment. In the documentation for the user, a statement shall be included drawing attention to the fact that there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

Consignes de sécurité

Examinez et suivez les consignes de sécurité ci-dessous :

• N'installez pas l'instrument dans un endroit humide comme une serre ou un incubateur pour éviter un risque de choc électrique. Si de l'eau ou tout autre matériau pénètre dans l'instrument, l'adaptateur, ou l'entrées d'alimentation, débranchez le cordon d'alimentation et contactez un technicien de service. Pour l'environnement d'exploitation, reportez-vous aux spécifications du produit.

• Ne touchez pas la fiche ou le cordon d'alimentation principale avec les mains mouillées.

• Assurez-vous toujours que la tension d'entrée d'alimentation correspond à la tension disponible dans votre endroit.

• Cet instrument est refroidi à l'air de sorte que ses surfaces peuvent devenir chaudes pendant le fonctionnement.

• Lors de l'installation de l'instrument, laisser un espace de plus de 10 cm (4 pouces) autour de cet instrument et ne placez aucun objet entre l'appareil et le mur.

• N'installez pas l'instrument sur une pente ou un endroit soumis à des vibrations, ce qui induit le risque de dysfonctionnement ou d'endommagement de l'instrument.

• N'insérez jamais aucun objet dans les orifices d'aération de l'instrument, car cela pourrait entraîner un choc électrique, des blessures chez les utilisateurs et des dommages d'équipement.

• Branchez le cordon d'alimentation fermement dans la prise murale et l'adaptateur secteur aussi.

• ur éviter un risque potentiel de commotion électrique, assurez-vous que le cordon d'alimentation est correctement mis à la terre.

• Assurez-vous de positionner l'instrument de telle sorte qu'il soit facile de débrancher l'instrument.

• Eteignez l'instrument avant de débrancher le cordon d'alimentation et / ou de déplacer l'instrument.

• Si l'instrument est cassé ou qu'il soit tombé, débranchez le cordon d'alimentation et contactez un technicien de service. Ne démontez pas l'instrument et la garantie sera annulée en cas de démontage.

• Utilisez uniquement les accessoires autorisés (l'adaptateur, le cordon d'alimentation, et le lecteur USB).

WARNING

Le produit de classe A est conçu pour l'utilisation dans un environnement industriel. Dans la documentation de l'utilisateur, la déclaration doit être incluse pour attirer l'attention sur le fait qu'il peut y avoir des difficultés potentielles pour assurer la compatibilité électromagnétique dans d'autres environnements, en raison des perturbations rayonnées et conduites par l'électricité.

Safety symbols

The following symbols are found on the instrument and this document. Always use the equipment in the safest possible manner.

Symbol	Meaning
	Caution & Warning
	Protective earth (Ground)
CE	This instrument and consumables conforms to the EC declaration of conformity.
FCC Compliance	This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.
	WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE.
	This product conforms to UL61010-1/CSA C22.2 No. 61010-1 "Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements." Instruments bearing the TUV symbol are certified by TUV SUD America Inc to be in conformance with the applicable safety standards for the US and Canada.

Technical support

Visit the our Website at **www.nanoentek.com** for:

- Technical resources, including manuals, FAQs, etc.
- Technical support contact information
- Additional product information and special offers.

For more information or technical assistance, please call or email.

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EVE™ PLUS

NESMU-EVE2-001E (V.2.5)

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CE

EVE™ HT

User Manual

NanoEntek

EVE™ HT User Manual

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The information in this user manual is described as accurately as possible.

Firmware and software changes and updates may change without prior consent or notification.

Document number: **NESMU-EVEHT-001E (V.0.4)**

Revision history: V.0.0 MAR 2022

V.0.1 JAN 2023

V.0.2 FEB 2023

V.0.3 OCT 2023

V.0.4 MAY 2025

Table of Contents

General Description

EVE™ HT is an automated multi-cell counter designed to count number of cells and measure viability of cells using trypan blue exclusion method. EVE™ HT uses automated image acquisition and proprietary image analysis method to quantify cell numbers and viabilities.

EVE™ HT requires only 20 µL of samples to run measurements. EVE™ HT Counting Plate is a disposable plate with 48 channels which allows EVE™ HT can measures 48 samples simultaneously.

EVE™ HT can count a wide variety of eukaryotic cells. EVE™ HT also measures the distribution of cell size measured under bright field imaging. EVE™ HT offers advanced algorithm to handle irregular shaped cells and declustering algorithm to handle clumped cells.

EVE™ HT has little user-to-user variations and it provides consistently accurate results regardless of whoever the user may be.

EVE™ HT offers optional “21 CFR part 11 module” to safeguard all the records and data in compliance to the FDA requirements.

Components

EVE™ HT is shipped with the following components.

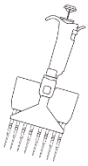
Upon receiving the instrument, please check that all items listed below are included in the shipment. If any of the items are missing or damaged, contact your local distributor or sales@nanoentek.com.

EVE™ HT

1 EA

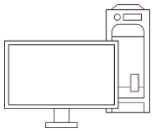
Multi pipette

1 EA



Operating desktop

1 SET



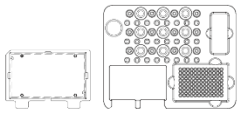
EVE™ HT Counting kit

1 EA

EVE HT COUNTING KIT

EVE™ HT Accessories

Preparation plate (optional)
QC plate (optional)



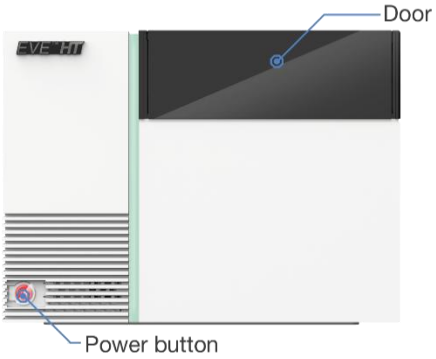
QC PLATE
(Low/Middle/High)

PREPARATION PLATE

EVE™ HT 21 CFR Part 11 software (optional)

Front View

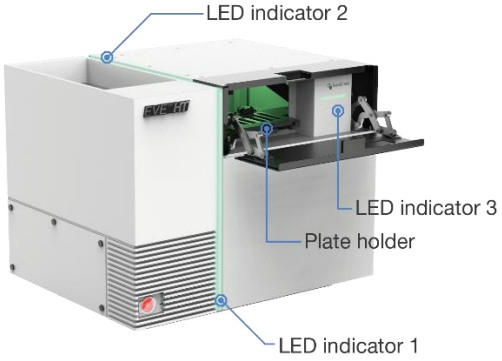
Front view showing various parts of EVE™ HT.



Part name	Description
Power button	Powering on/off
Door	Instrument door

Upper Side View

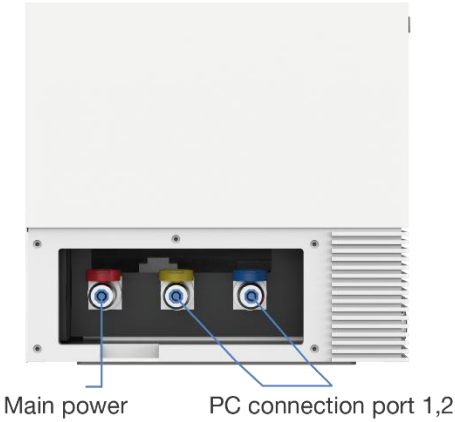
Upper side view showing various parts of EVE™ HT.



Part name	Description
LED indicator	Product status display
Plate holder	Holder for plate when inserted/ejected

Left Side View

Left side view showing various parts of EVE™ HT.



Part name	Description
Main power	Connection port for electrical outlet
PC connection ports	Connection ports for PC

Environmental Requirements

⚠ **CAUTION**

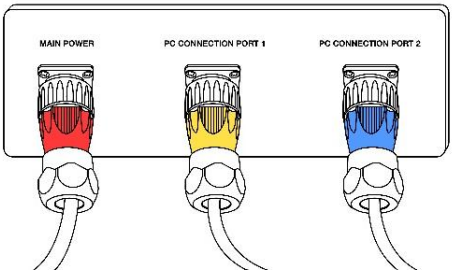
At low temperature (≤10 °C), allow the instrument to warm up for 10 minutes at ambient temperature before use.

To ensure correct operation and stable performance, install EVE™ HT in a proper location by complying with the followings:

- Use at room temperature.
- Do not expose the instrument to direct sunlight.
- Do not expose the instrument to direct or continuous vibration.
- Do not expose the instrument to intense magnetic or electromagnetic fields.
- Do not install the instrument in high-humidity environment.
- Installation should be free corrosive gases or other corrosive substances.
- Minimize contact with dust or airborne particles.
- Secure at least 10 cm (4 inches) of free space around the instrument for the proper airflow.
- Do not place objects on top of this instrument.
- Connect EVE™ HT power cord to wall outlet directly.

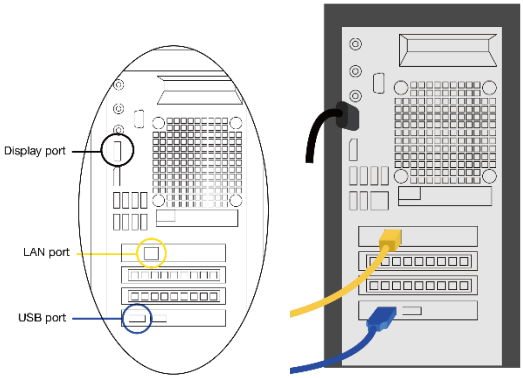
Powering on and Installation

1. Connect the EVE™ HT with the color-coded power cord and connection cords of PC.



2. Connect the EVE™ HT to PC with LAN and USB cable.

3. Connect the PC to a PC monitor using a monitor cable. Then, connect the power cords to the PC and PC monitor.



⚠ **CAUTION**

- ***Do not tilt the instrument too much when connecting the power cord.***
- ***Do not move the instrument after connected to the power cord.***

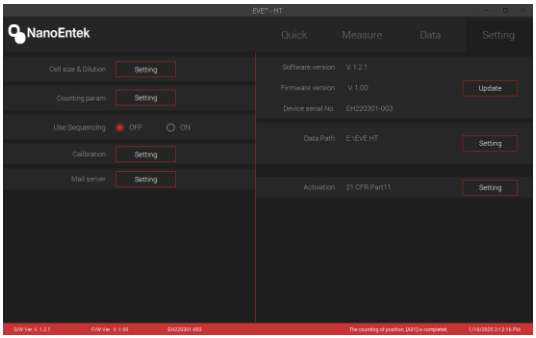
4. Turn on EVE™ HT instrument.
5. Turn on the PC and run EVE™ HT main software.

⚠ **CAUTION**

If the error code occurs during the program initializing process, turn off the instrument and the PC. Repeat Step 4 and 5 above. If the same error message appears repeatedly, contact your local distributor or sales@nanoentek.com.

Instrument Settings

Press '**SETTING**' from the startup screen. The setting menu allows you to set up the followings.



1. Click '**Calibration**' to calibrate the instrument image background level.

a) Calibration sample preparation

① Mix **20 µL of culture media** and **20 µL of trypan blue** thoroughly.

② Load **20 µL of the mixture** into one channel in an EVE™ HT Counting Plate.

③ Insert the counting plate into the plate holder of the instrument.

b) Calibration setting

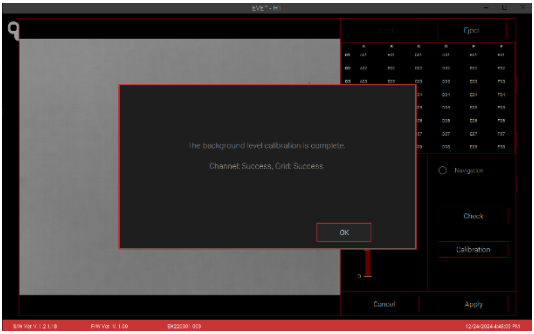
① Select '**Setting**' tab.

② Click **Calibration 'Setting'** button.

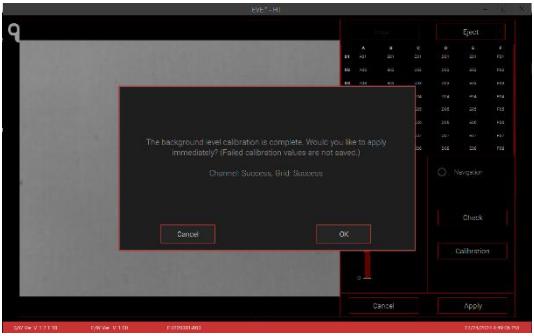
③ Click **'Insert'** button.

④ Select the **wells** loaded with the mixture

⑤ Click **'Check'** button.



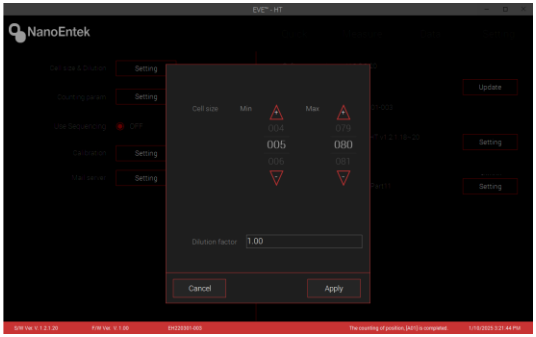
⑥ Click **'Calibration'** button.



⑦ After finish the calibration, click **'OK'** and **'Apply'** button.

2. Set the **minimum and maximum cell size**.

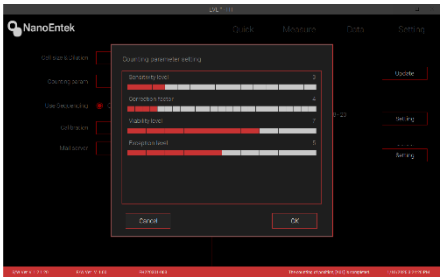
3. Set the **dilution factor** according to the test.



☛ ***NOTE***

Mix ratio (sample 20 µL + Trypan Blue 20 µL) is already applied so do not apply to dilution factor.

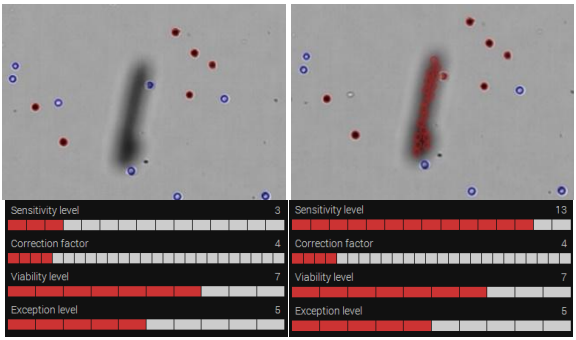
4. Set the **Counting parameter.**



It is highly recommended that users use default counting parameters. However, depending on cell types and media conditions, one may need to adjust these counting parameters.

① Sensitivity level

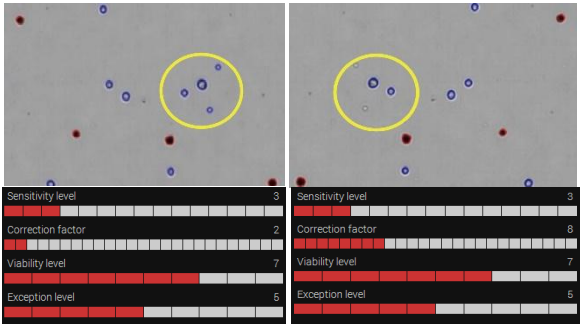
As this level gets lowers, it becomes easier to remove the effects of debris.



< Sensitivity 3> < Sensitivity 13>

② Correction factor

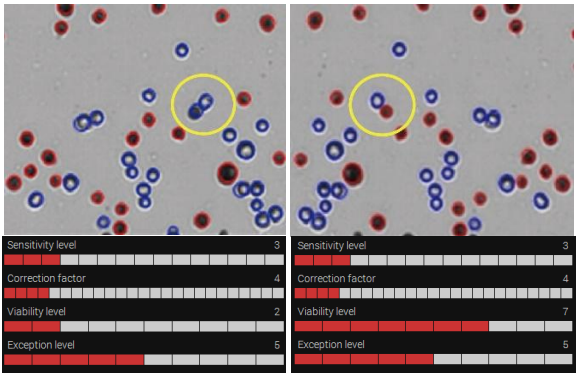
As this factor gets lower, the software becomes more sensitive to pick up vague objects.



<Correction factor 2> <Correction factor 8>

③ Viability level

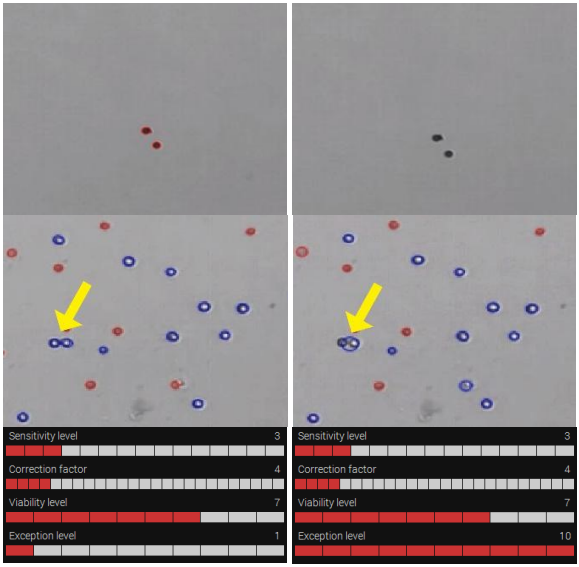
As this level gets higher, marginally dark objects won't be counted as dead cells.



<Viability level 2> <Viability level 7>

④ Exception level

As this level gets lower, the software becomes more sensitive to pick up small objects. This is useful for counting aggregation samples or small cells.



<Exception level 1> <Exception level 10>

Instrument Settings

5. Use Sequencing
   ① Default setting for assigning sequential number after the sample name.

6. Update to install new **software** or **firmware** as they become available.
   ① Connect the **USB** with the update file to computer.
   ② Click **'Update'** button in setting tab.

7. Data Path
   ① Click Data Path **'setting'** button in setting tab.
   ② Set the data storage path.

8. Mail server
   **Do not change Mail settings.*

9. 21 CFR Part11
   ① 21 CFR PART11 is a separately purchased function. A detailed description is at the end of the manual.

Recommended Actions

To obtain the best results, follow these recommendations:

1. Sample

① Wear protective gloves while handling samples.

② For accurate results, ensure that samples are well mixed.

③ Also, allow loaded samples to settle for **2 minutes** before Focus setting.

2. Trypan blue

① Warm up to room temperature before use in order to avoid debris.

3. EVE™ HT Counting Plate

① Keep the Counting Plate in the carriage and use on a clean table to prevent dust from sticking.

② Do not touch any surfaces except for the handles

③ Ensure that the sample fills up entire channel.

④ Do not tilt the plate after loading the sample.

⑤ Do not insert the plate upside-down as this may cause liquid to flow into the instrument and cause damage.

⑥ Do not re-load the sample into used channel. One can use same plate multiple times as long as new channel(s) are used each time.

⑦ Make sure to push the plate all the way in.

4. Instrument

① Use EVE™ HT at room temperature only (20 ~ 25 °C).

② Turn on the instrument before the EVE™ HT software.

③ Do the background calibration when using trypan blue with a new lot number. (Refer to page 11).

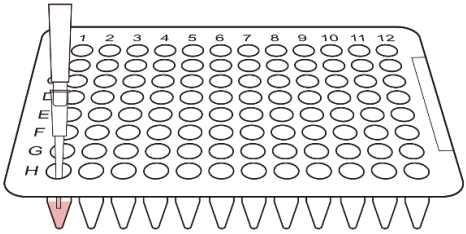
④ Make sure the door and plate holder are closed.

Sample Test

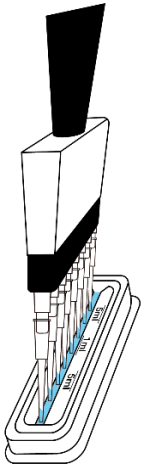
Sample Preparation

Instruction is provided in this chapter for preparing the sample using EVE™ HT trypan blue stain and disposable EVE™ HT Counting Plate.

1. Prepare cell suspensions either in growth media or PBS
2. Thoroughly **mix the cell pellet** by vortexing.
3. Dilute cell solutions so that expected final concentration will be between 1×10^4 and 1×10^7 cells/mL.
4. Load **20 µL of well-mixed sample** into a mix well plate. Small volume PCR plates are included in EVE™ HT kit to be used as mix well plates.



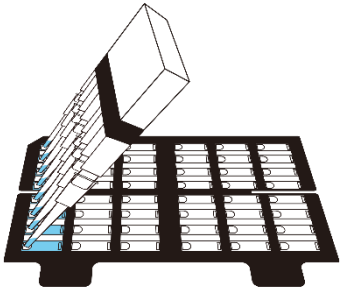
5. Dispense enough amount of **trypan blue** to a reservoir. Disposable reservoirs are included in EVE™ HT kit.
6. Add **20 µL of trypan blue** to the sample-loaded well using multi pipette and **mix the sample and trypan blue by pipetting up and down**. A 8-channell multiwell pipette is included in each EVE™ HT.



Sample Test

Sample Preparation

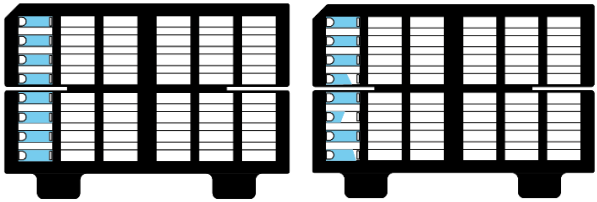
7. Load **20 µL of sample mixture** into the EVE™ HT Counting plate.



☞ ***NOTE***

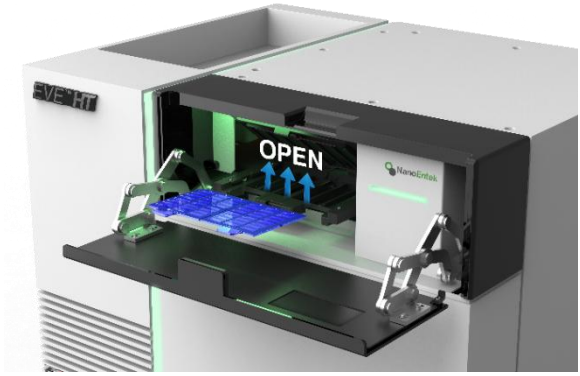
EVE™ HT can analyze cell concentrations of 1 x 10^4 to 1x 10^7 cells/mL.

Correct and incorrect example of loaded sample

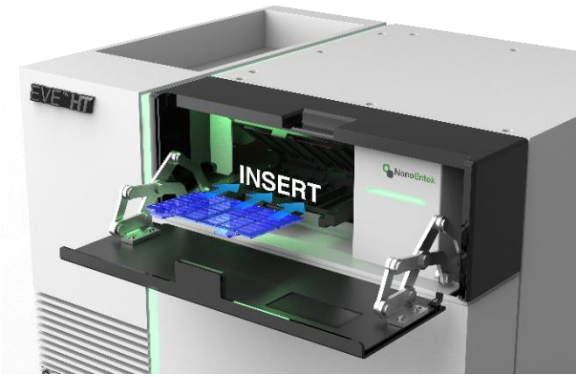


Correct Incorrect

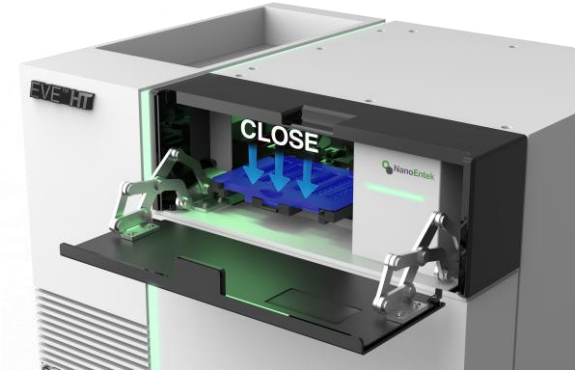
8. Open the EVE™ HT door and press **plate holder** cap to open the plate.



9. Insert the **EVE™ HT Counting plate** loaded with sample into the plate holder.



10. First close the **plate holder**, then close the **door**.



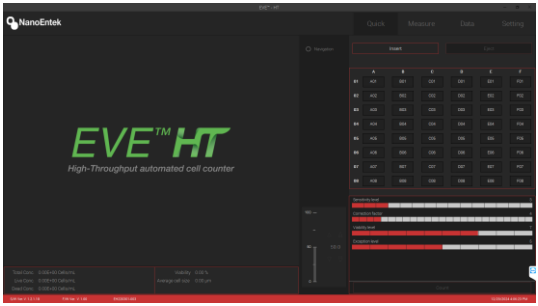
⚠ ***CAUTION***
Allow sample to settle for '2 minutes' after inserting the EVE™ HT Counting Plate into the instrument.

⚠ ***CAUTION***
Make sure to put the plate all the way in when inserting the plate.

⚠ ***CAUTION***
Make sure to properly close the door and plate holder cover before operating.

Sample Test

Quick Count



Quick count tab

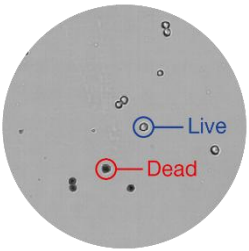
The quick count function captures the displayed image in real time and counts one frame.

1. Click the **'Insert'** button.
2. After finish the insert the plate, click the **sample loaded well**.
3. Set the **focus** following the focus guide.
4. Adjust the **focus** using the **'focus'** buttons.

* Fine focus: Hold **'Shift** or **Control'** button and **scroll the mouse wheel** over the image for adjustment.

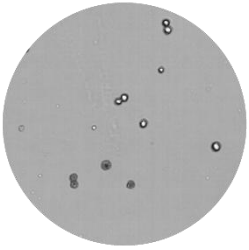
* Focus example

a) Good focus



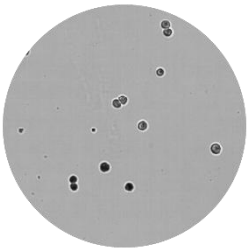
▶ Live cells have bright centers and dark edges.
▶ Dead cells have a uniformly blue color throughout the cell with no bright centers.

b) Bad focus



▶ Dead cells have bright centers and blurry boundaries.

c) Bad focus2



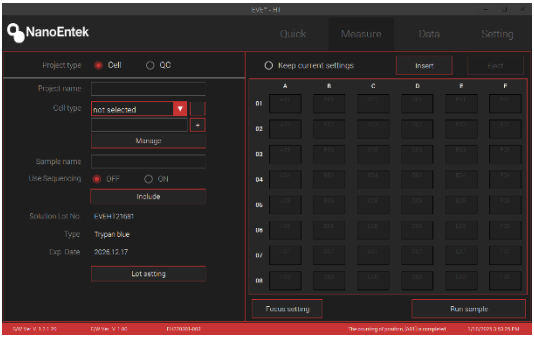
▶ Live cells with dark centers are counted as the dead cells.

5. Check the **counting parameter**.
6. Click the **'Count'** button.
7. The results are displayed on the **Quick Count section** from the **Data** menu.

Sample Test

Measure

This section provides procedures and tips for cell counting using EVE™ HT.



Measure tab

1. Click 'Measure' tab.

2. For project type, select 'Cell' and enter 'Project name'.

3. Select 'Cell type' and enter 'Sample name'.

*** Cell type**

a) Make the new cell type.

① Enter the **name of cell type**.

② Click the **'+'** button.

③ Set the **'Cell size'** and **'Dilution factor'**

④ Click the **'Apply'** button.

b) Using the previous cell type

① Click the **'∇'** button.

② Select the cell type.

c) Delete the cell type

① Click the **'∇'** button.

② Select the **cell type**.

③ Click the **'-'** button.

④ Click the **'OK'** button.

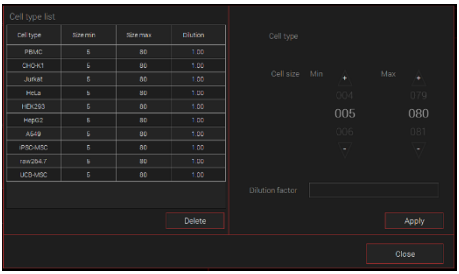
d) Manage the cell type.

① Click the **'Manage'** button.

② Select the **cell type**.

③ Set the **cell size** and **dilution factor**.

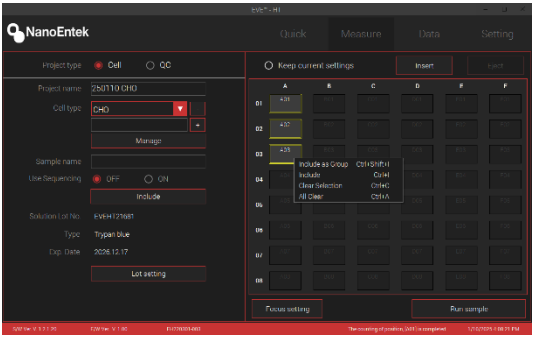
④ Click the **'Apply'** button.



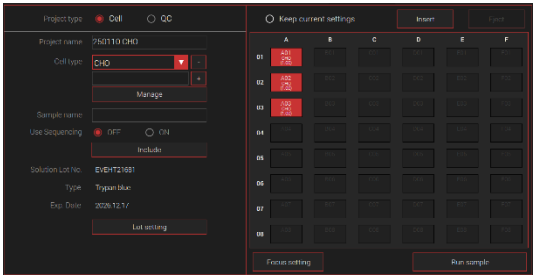
4. Select the wells loaded with samples. For individual selection, click each well you want to measure. For group selection, left click and drag.

5. Click 'Include' button or choose 'Include' option from 'Select' menu by right click. Then, make sure the selected wells are displayed in red.

6. Repeat Step 3 to 5 for remaining wells.



Before include

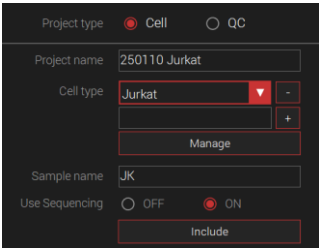


After include

*** After entering the sample name, set 'Use Sequencing' to 'ON', and click 'Include', a sequential number is assigned to the sample name.**

Sample Test

Measure

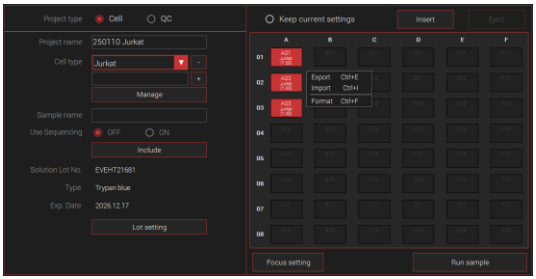


*** Use the previous Well setting.**

A. Click the 'Keep current setting' on measure tab.

B. Well setting format

User can edit the well setting in excel file.



a) Export

① Click the **'right'** button of the mouse in the Well Setting window.

② Click the **'Format'** or **'Export'** and enter the file name and pathway.

Sample	Include	Group	Name	Cell type	Size min	Size max	Dilution
A01	X				5	80	1
A02	X				5	80	1
A03	X				5	80	1
A04	X				5	80	1
A05	X				5	80	1
A06	X				5	80	1
A07	X				5	80	1
A08	X				5	80	1
B01	X				5	80	1
B02	X				5	80	1
B03	X				5	80	1
B04	X				5	80	1
B05	X				5	80	1
B06	X				5	80	1
B07	X				5	80	1
B08	X				5	80	1

Format

Sample	Include	Group	Name	Cell type	Size min	Size max	Dilution
A01	O		A01	Jurkat	5	80	1
A02	O		A02	Jurkat	5	80	1
A03	O		A03	Jurkat	5	80	1
A04	X						
A05	X						
A06	X						
A07	X						
A08	X						
B01	X						
B02	X						
B03	X						
B04	X						
B05	X						
B06	X						
B07	X						
B08	X						

Export

b) Edit

① Open the **excel file**.

② **Edit** the file (Include, Group, Name, Cell type, Size min, Size max, Dilution) and save.

c) Import

① Click the **'right'** button of the mouse in the Well Setting window.

② Click the **'Import'** and **select** the excel file.

☞ ***NOTE***

If the parameters in the Excel file do not match the values of the Cell type, the values from the Excel file will take priority, and the Cell type parameters will be updated accordingly.

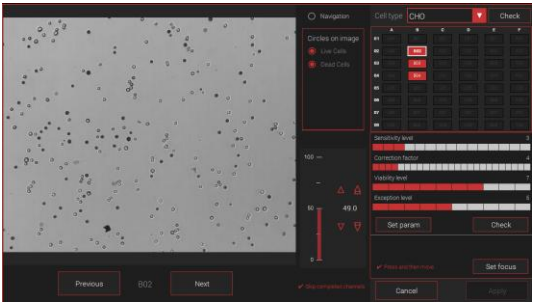
7. Adjust the focus using the 'Focus Setting'.

☞ ***NOTE***

Load the Counting plate in the plate holder and perform 'Focus setting' after 2 minutes.

8. Set the 'counting parameter'. Refer to the page 13-14.

9. Click 'Check' button to check live and dead cells at the current focus location. Refer to the page 20-21.

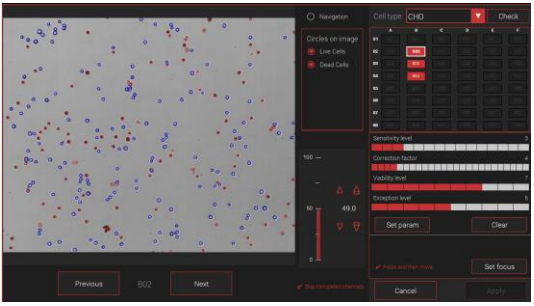


Focus setting

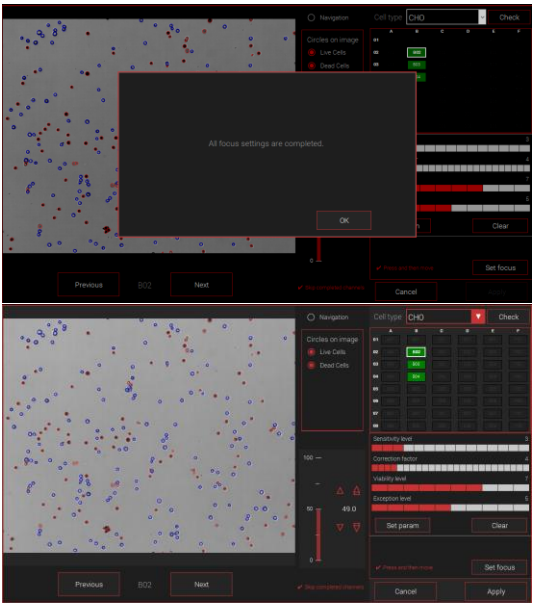
Navigation	Navigation on/off.
Circles	Circle (Live Cells / Dead Cells) on/off
Cell type	Select the cell type to set focus on and check it.
Set param	Set parameters.
Check	Count the cells on the current screen using the Quick count method.
Set focus	Apply the focus value.
Press and then move	After applying the focus setting, move to the next channel.
Skip completed channels	Channels that have been focused will be skipped.
Previous/Next	Previous/Next move buttons

Sample Test

Measure



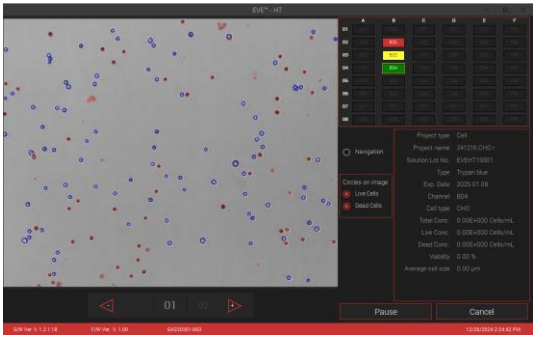
Check



Set Focus

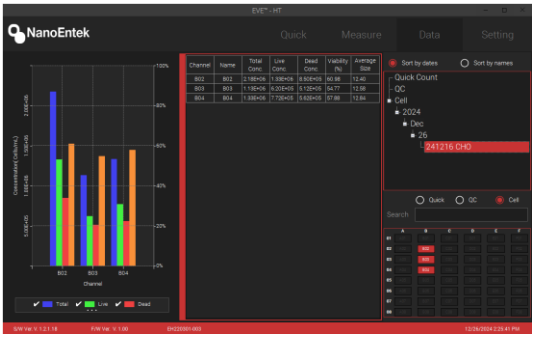
10. After confirming the desired settings, click ‘Set Focus’ and ‘Apply’ button.

11. Click the ‘Run sample’ button.



Running

* During measurements, results for wells already counted can be checked while counting other remaining wells.



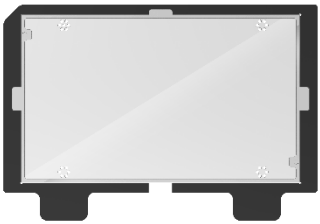
12. The results are displayed on the Cell section from the Data menu.

Quality Control

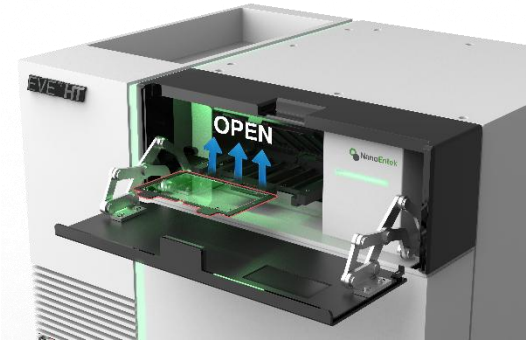
QC plate Preparation

This is for quality control using **QC plate**. Follow the instruction below only if necessary.

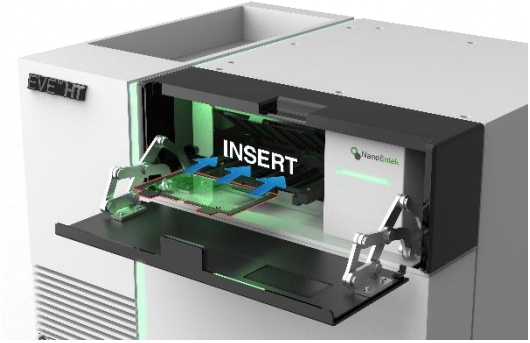
1. Prepare the **QC plate**.



2. Open the EVE™ HT **door**.
3. Push the **plate holder cap** to open up the plate holder.



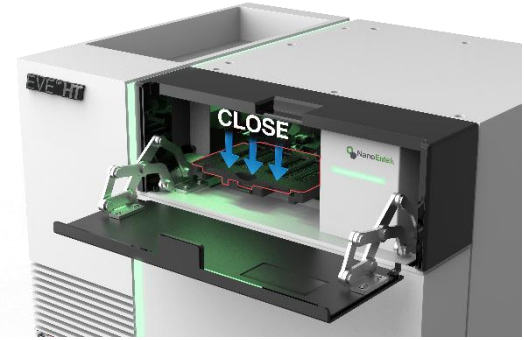
4. Insert the **QC plate** into the plate holder of the instrument.



⚠ ***CAUTION***

Make sure to push the plate all the way in.

5. First close the **plate holder**, then close the **door**.



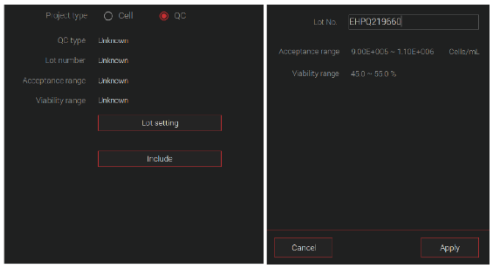
⚠ ***CAUTION***

Make sure the door and plate holder are properly closed.

Quality Control

QC plate Run

1. Select menu & QC plate Lot setting



① Click the **'Measure'** tab.

② Select the **'QC'** on the project type.

③ Click the **'Lot setting'**.

④ Click the **'New'** button for creating the new lot.

⑤ Enter the **'Lot number'**.

* The lot number can be found on the plastic package label.
* The last lot used will be automatically applied from the next time.

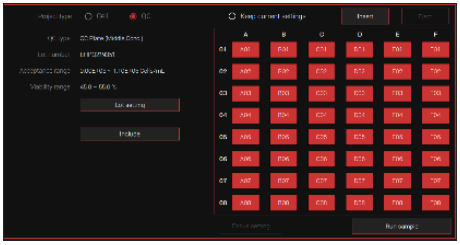
⑥ Click the **'Apply'** button and check the **'Acceptance range'**.

2. Using previous lot

① Click **'Lot Setting'** button and find the lot number from **'Search'** tab.

② Select the lot you want and click **'Apply'** button.

3. Well setting



① Select all 48 channels, then click **'Include'** button or select **'Include'** option from **'Select'** menu by right click. Make sure the selected wells are displayed **in red**.

② Well setting can be edited from the **'Select'** menu.

③ QC Plate does not require a focus setting procedure because the auto focus function is applied.

4. Run Sample

① Click the **'Run sample'** button.
* During measurements, results for wells already counted can be checked while counting other remaining wells.
② The results are displayed on the **QC section** from the **Data** menu.

5. Data Analysis

Select the '**Data**' tap to check the results. The result of performance test should meet the '**Acceptance range**' in the QC Plate.

- **Acceptance range:**

· **Low level** (Cat. no. EHPQ-001)
Cell concentration : 6.96 x 10^4 ~ 1.04 x 10^5 cells/mL
Viability : 25.0 ~ 35.0 %

· **Middle level** (Cat. no. EHPQ-002)
Cell concentration : 9.00 x 10^5 ~ 1.10 x 10^6 cells/mL
Viability : 45.0 ~ 55.0 %

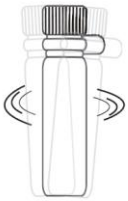
· **High level** (Cat. no. EHPQ-003)
Cell concentration : 4.50 x 10^6 ~ 5.50 x 10^6 cells/mL
Viability : 75.0 ~ 85.0 %

Quality Control

QC bead Preparation

This is for quality control using **Bead Solution**. Follow the instruction below only if necessary.

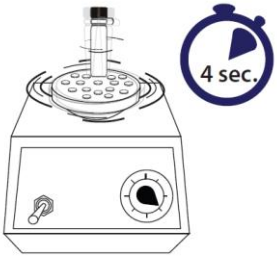
1. Let the **Bead Solution** come to room temperature for up to **10 minutes** before use.
2. Shake bottle vigorously or vortex briefly for 5 seconds before use.



[Shake bottle vigorously]

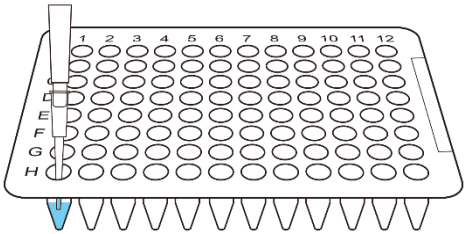
[Vortexing]

3. Vortex again for 4 seconds and pipette immediately.

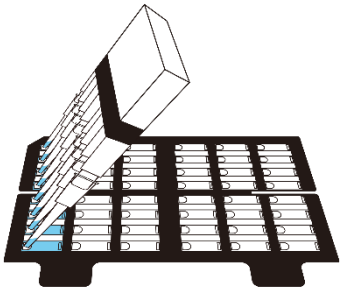


[Vortexing]

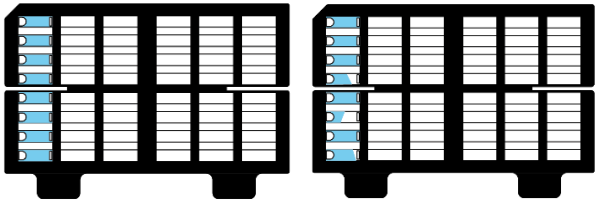
4. Transfer **20 µL of calibration bead** to the Mix Well Plate, add **20 µL of trypan blue**, and mix well by pipetting up and down.



5. Load **20 µL of mixture** into each channel of EVE™ HT Counting plate.



Correct and incorrect example of loaded sample



Correct Incorrect

6. Open the EVE™ HT **door**.
7. Push the **plate holder cap** to open up the plate holder.
8. Insert the **plate** into the plate holder of the instrument.

⚠ ***CAUTION***
Make sure to push the plate all the way in.

☞ ***NOTE***
Allow beads to settle for '1 minute' after inserting the EVE™ HT Counting plate to the instrument.

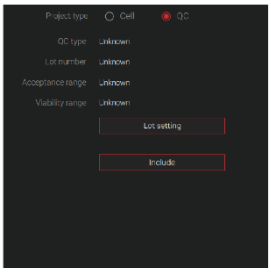
9. Close the **plate holder** first then the **door**.

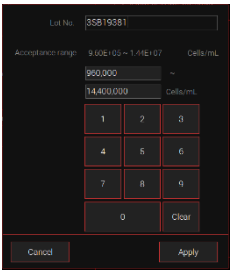
⚠ ***CAUTION***
Make sure the door and plate holder are properly closed.

Quality Control

QC bead Run

1. Select menu & QC bead Lot setting





① Click the **'Measure'** tab.

② Select the **'QC'** on the project type.

③ Click the **'Lot setting'** button.

④ Click the **'New'** button for creating the new lot.

⑤ Enter the **'Lot number & Acceptance range'**.

* The lot number & acceptance range can be found on the plastic package label.

* The last lot used will be automatically applied from the next time.

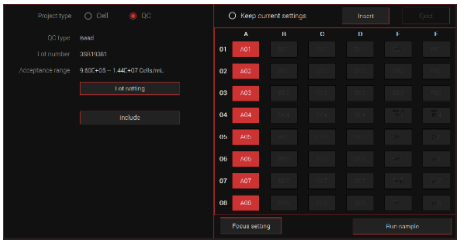
⑥ Click the **'Apply'** button and check the **'Acceptance range'**.

2. Using previous lot

① Click **'Lot Setting'** button and find the lot number from **'Search'** tab.

② Select the lot you want and click **'Apply'** button.

3. Well setting



① Select the **wells** loaded with samples. Click or drag to select the wells you want to measure.

② Click **'Include'** button or select '**Include'** option from **'Select'** menu by right click. Make sure the selected wells are displayed in red as shown.

③ Well setting can be edited from the **'Select'** menu.

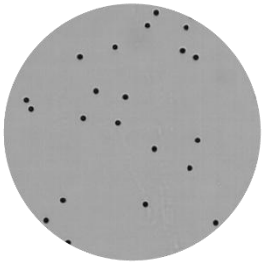
④ Click **'Focus Setting'**.

4. Focus setting

① Adjust the focus using the focus buttons.

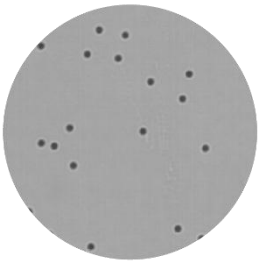
* Fine focus: Hold **'Shift'** or **'Control'** button and **scroll the mouse wheel** for adjustment.

*** Focus example**



Good focus

▶Beads have a uniformly dark color throughout the beads with no bright centers.



Bad focus

▶ Beads have blurry boundaries.

② Click **'Set Focus'** button.

③ Click **'Apply'** button.

5. Counting and getting result

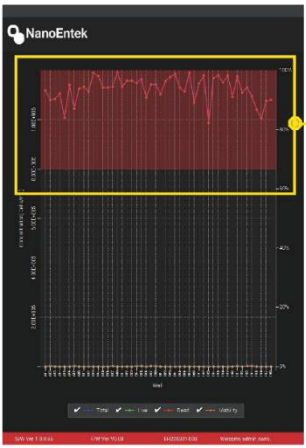
① Click **'Run sample**' button.

② During measurements, results for wells already counted can be checked while counting other remaining wells.

③ The results are displayed on the **QC section** from the **Data** menu.

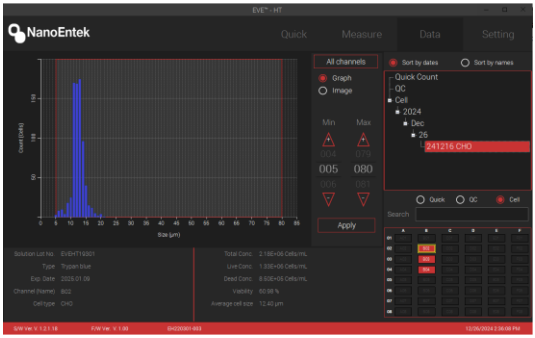
6. Data Analysis

The result should be **within the range on the label** of the QC bead bottle.
(e.g. 8.0E+05 ~ 1.2E+06/mL)

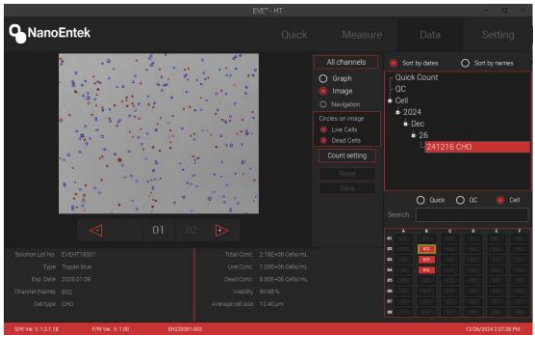


Data

Data menu allows users to review the raw data including counting results and images from each well. Reviewing, editing, saving and exporting the data can be done.



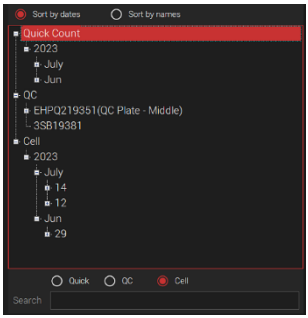
Data menu (Well graph)



Data menu (Well image)

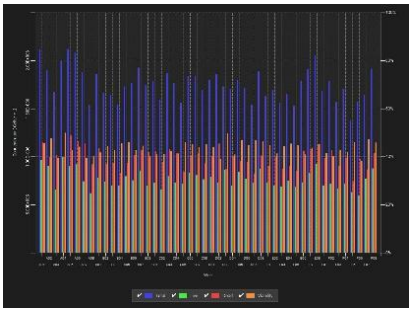
1. Data list

When counting is complete, all the information is automatically stored in the data list. This list can be sorted by either date or name.



Quick Count	Data list taken from the Quick menu.
QC	Data list taken from the QC mode (Project type).
Cell	Data list taken from the Cell mode (Project type).
View type	It can be viewed by two criteria, date or name.
Search	Search the data in each section.

2. Graph

Lot graph in QC mode	Period of date can be set. (Day, Month, Year or Index)
	• Maximum of 48 counting results will be displayed on the graph and the data list. • The graph and table of well are linked. When you select the data on the graph, the corresponding well will be displayed in the table. When you double click the well in the table, the corresponding well will be displayed.
Plate graph in QC and cell mode	

Graph in well

- Value for minimum and maximum cell size can be set in **Size graph**.
- Minimum and maximum cell sizes are used to determine the low and high ranges of cell size for measurement.

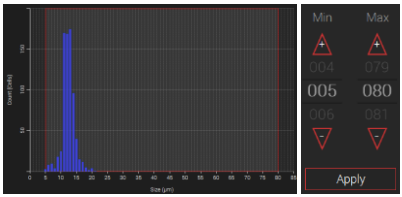
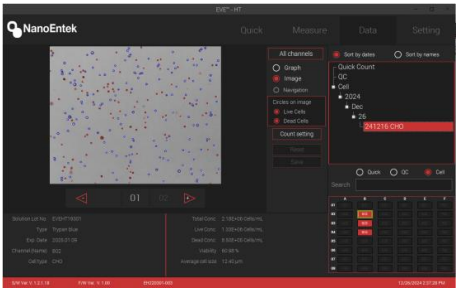
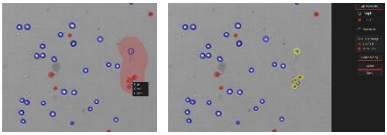


Image in well

Users can edit the following functions through the captured image:



- Circle (Live Cells / Dead Cells) on/off
- Navigation on/off
- Image editing:



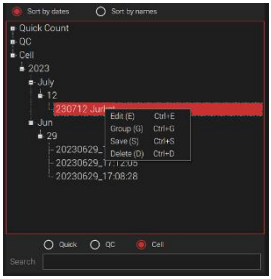
① Right-click on the circle to go to menu. Right-click and drag images for multiple selection.

② Click live, dead or debris.

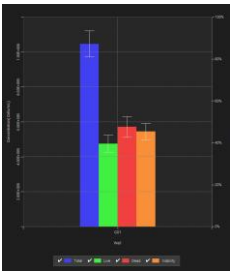
③ Click '**Save**' button to apply or '**Reset**' button to cancel.

④ Change the Counting parameter. Refer to the page 13-14.

3. Edit function



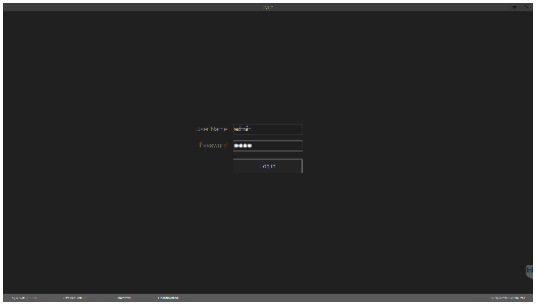
- **Right-click** on each project or well to go to menu.

Edit	Rename the project name. Set the Size gating and dilution factor. Set the Acceptance range is only possible in QC data. ✓ Edit can be modified in selected multi-wells.
Group	To re-edit group setting, select **Group**. 
Save	To save project, select **Save**. ✓ The data type and pathway can be selected.
Delete	To delete project, select **Delete**.

21 CFR Part 11

Log in

When activating 21 CFR Part11, the user must log in on the first screen.

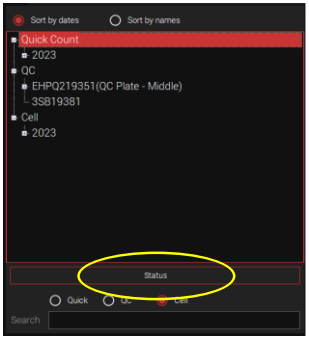


*Log in from login screen. (default ID/PW : admin/0000)
*Log out is in the User tab.

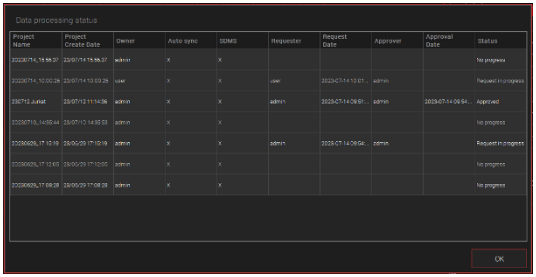
21 CFR Part 11

Data Status

Users can check data status upon 21 CFR Part11 activation.



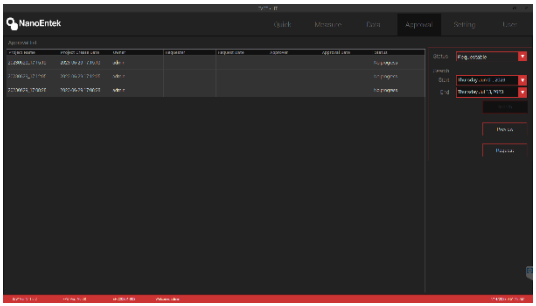
Data list



Data status

21 CFR Part 11

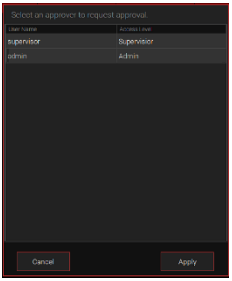
Approval



Approval

Requestable

The list shows data that can be requested for approval.
1. Select the data to get approve.
2. Click the '**Request**' button.

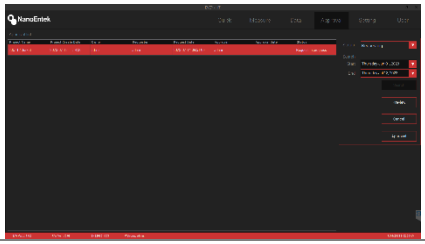


3. Select an approver to request approval.
4. Click the '**Apply**' button.

Requesting

The requesting data are displayed.
It is possible to '**Cancel**' the request for approval.

The approver can approve the request in Requesting tab. The approval no need to log in to the approval ID.



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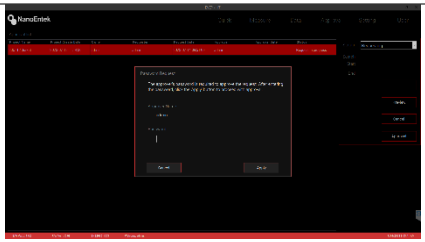
21 CFR Part 11

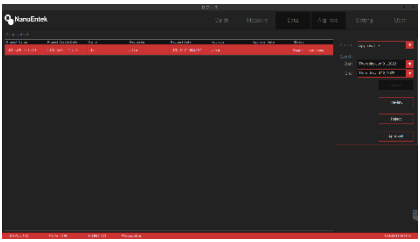
Approval



1. Select the Data in list
2. Click the ‘Approval’
3. Enter the password.

The approvable data are displayed on this tab.
It is possible to ‘**Reject**’ or ‘**Approval**’.

Approvable

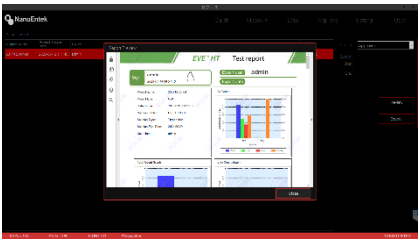


Approved

The approved data is listed.
It is possible to ‘**Export**’ the Approved data.

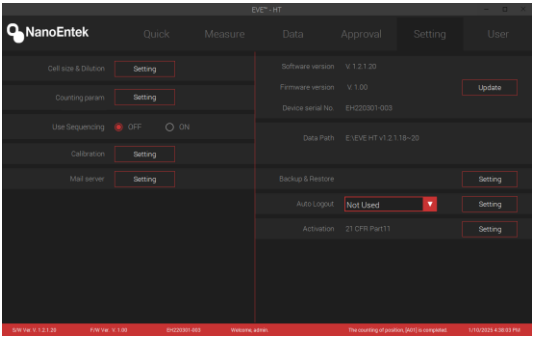
Preview

All status data can be previewed in PDF format.



21 CFR Part 11

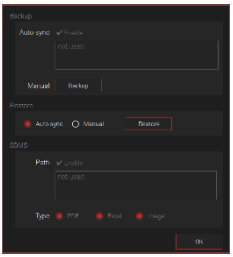
Setting



Setting tab after 21 CFR Part 11 activation

1. **Backup & Restore**

① Click the Backup & Restore **'Setting'** button.



② To enable automatic backup, click the Auto sync 'Enable', set the backup data path.

③ Click the Manual **'Backup'** button, save the backup data at the current point in time.

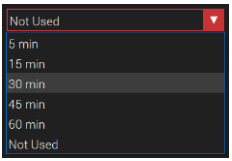
④ The Restore function provides two options. You can back up based on the backup data saved by Auto sync or the backup data saved by Manual.

⑤ Click the SDMS Path 'Enable', set the SDMS data path.

⑥ Select the desired type of SDMS data.

2. **Auto Logout**

① Click the **'▽'** button

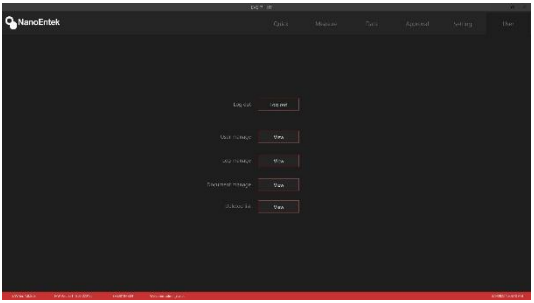


② Select the auto logout limit time.

③ Click the Auto Logout **'Setting'** button.

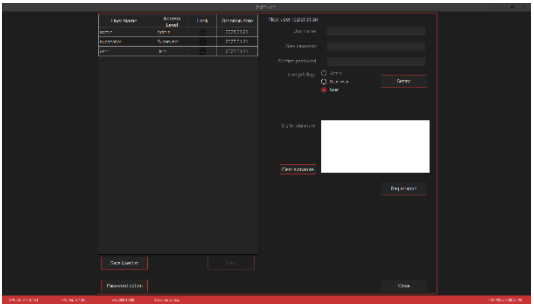
21 CFR Part 11

User manage



User tab

1. User management
Click the User manage **'View'** button.

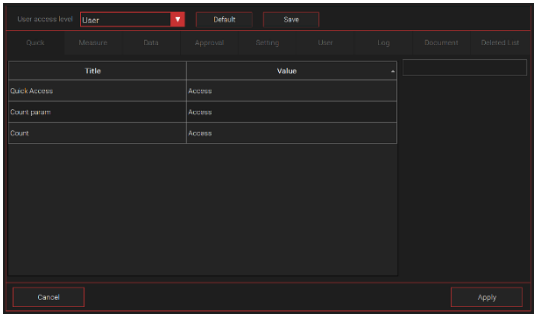


a) New user registration.

① Enter the user **ID** and **PW**.

② Click the User privilege **'Setting'**.

③ Set the User privilege in each menu.



* User and Supervisor **default permission** settings can be changed.

- ✓ *Set the privilege and click the **'save'** button.*
- ✓ *See the 21 CFR part 11 Supplement for default settings.*

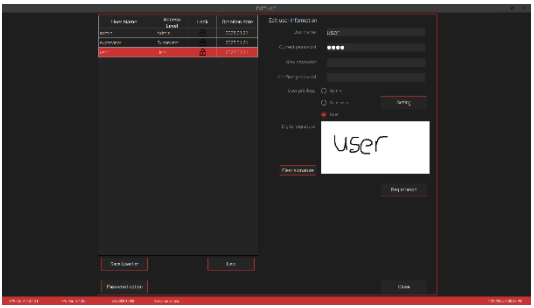
④ Set the User and Supervisor permission.

⑤ Click the **'Apply'** button.

⑥ Enter the signature and Click the **'Registration'**

b) Edit the user option

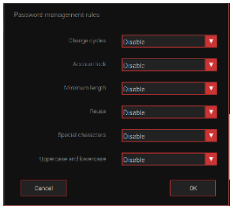
① Select the **user** in user list.



② Do the same process in **Creating New user**.

③ Depending on the user's granted privileges, the account can be locked.

c) Password option



Set the password management rules.

① Change cycles
Disable, 30 days, 60 days, 180 days

② Account lock
Disable, ≥ 3 times, ≥ 5 times, ≥ 10 times, ≥ 15 times

③ Minimum length
Disable, ≥ 3, ≥ 5, ≥ 10, ≥ 15

④ Reuse
Disable, ≥ 30 days, ≥ 60 days, ≥ 180 days

⑤ Special characters
Disable, Enable

⑥ Uppercase and lowercase
Disable, Enable

d) Lock in user list

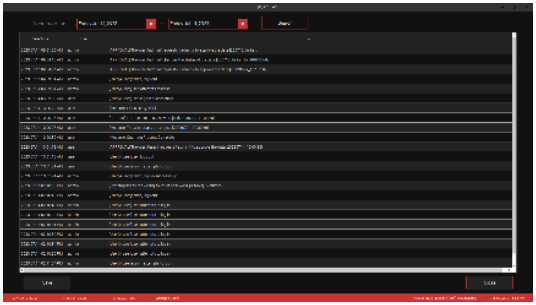
User Name	Access Level	Lock	Creation date
admin	Admin		2025.01.21
supervisor	Supervisor		2025.01.21
user	User		2025.01.21

User ID is locked when login fails. Lock icon turns red.
Click the button to unlock user ID and the button changes to grey.

21 CFR Part 11

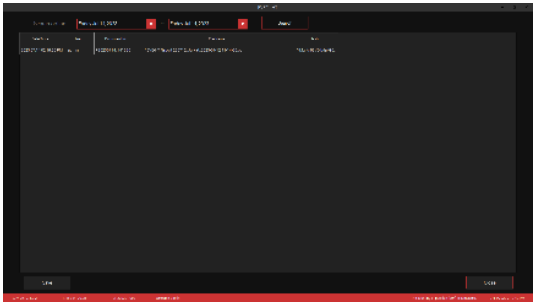
Log manage, Document manage, Deleted list

1. Log manage



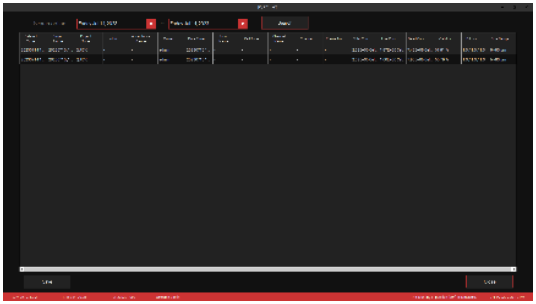
This '**Log manage**' allows you to view a list of instrument log.
* Every logs can be saved in CSV file.

2. Document manage



This '**Document manage**' allows you to check the documents made in the equipment.

3. Deleted list



This '**Deleted list**' allows you to view a list of deleted data.

** EVE-HT provides a comprehensive solution to comply with the requirements of the 21 CFR Part 11 rule.*

** Please see the appendix for more information on these features.*

Maintenance and Cleaning

Clean the surface of EVE™ HT with a damp cloth.

The EVE™ HT does not need regular maintenance. To troubleshoot problems with the instrument, contact technical support. Do not perform any repairs or service on EVE™ HT to avoid damaging the instrument.

Trouble Shooting

Inaccurate result

1. Low and high results

- EVE™ HT is designed to read samples from 1 × 10^4 cells/mL to 1 × 10^7 cells/mL.
- If your sample is out of this range, you may need to dilute the sample or add more cells and read the sample again.

2. Dilution factor

- Check the mixing ratio of Sample 20 µL + Trypan blue 20 µL.
  Mix ratio is already applied so do not apply to dilution factor.
- Apply sample dilution to the dilution factor.

3. Dust or bubble

- Check the surface of EVE™ HT Counting Plate.
- Be careful not to make any bubble when mixing and loading sample with a pipette.
- Put trypan blue to room temperature for warm up before use in order to avoid debris.
- Set the 'Counting parameter' before count in Focus setting window. Refer to pages 13-14.
- Remove any bubble and dust in the image after count using the image edit function Refer to pages 40.

4. Incorrect focus

- Set the correct focus. Refer to page 20-21.

5. Background calibration

- Too bright or too dark background affects the result.
- Do the Background calibration in the Setting tab, when using trypan blue with a new lot number. Refer to page 11.

6. Too big or too many clumpy cell

- Ensure the cells are not clumped.

7. Plate

- Push the plate all the way in.

Saving problems

1. E-mail

- Check the internet connection.

2. USB

- Check the storage path.

Error Message

Error Message

Error message	Solution
The EVE device is off. Please turn the EVE on and restart the EVE software. Please press the 'OK' button to finish the EVE software.	Turn on EVE™ HT prior to the EVE™ HT software.
Please insert a plate to use the EVE-HT.	Make sure put the plate all the way in.
Please close the holder to use the EVE-HT.	Close the cover of plate holder.
Please close the door to use the EVE-HT.	Close the door completely.

If the same error message appears repeatedly, contact your local distributor or sales@nanoentek.com.

Warranty

Under normal use, NanoEntek warrants that EVE™ HT shall be free from defects in material and workmanship for a period of one (1) year from the date of original purchase.
If any defects occur in EVE™ HT during this warranty period, NanoEntek will repair or replace the defective parts at its discretion without charge.

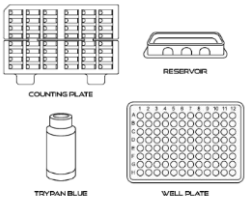
The following defects, however, are specifically excluded:
1. Defects caused by improper operation.
2. Repair or modification done by anyone other than NanoEntek or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than NanoEntek.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent or sample.

For your protection, EVE™ HT to be returned must be insured against possible damage or loss. NanoEntek will not be responsible for damage incurred during shipment of the defective instrument. It is recommended that you save the original packing material in which the instrument was contained. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service, please contact sales@nanoentek.com or your local distributor.

Technical Specifications

EVE™ HT	
Measuring range	1 x 10^4 ~ 1 x 10^7 cells/mL
Analysis time	< 3 minutes / 48 tests
Light	Bright
Number of channel	1
Cell size	5 ~ 80 μm
21 CFR Part 11	Available
Operation System	Windows 10 Enterprise LTSC
Size (W x D x H)	586 mm (W) x 461 mm (D) x 458 mm (H)
Weight	58 kg
Operation	
Temperature	5℃ ≤ Temperature ≤ 40℃
Humidity	20% ≤ Humidity ≤ 80%
Altitude	Altitude ≤ 2,000 m
Accessories	
Multi pipette	1 ea
EVE™ HT Counting kit	
Counting plate	15 ~ 30℃ (59 ~ 86℉)
Solutions	
Trypan blue	15 ~ 30℃ (59 ~ 86℉)
Storage temperature	
Counting plate	15 ~ 30℃ (59 ~ 86℉)
Trypan blue	15 ~ 30℃ (59 ~ 86℉)
Expiration Date	
Counting plate	2 years
Trypan blue	Check the reagent label 6 months after opening



Ordering information

Cat. No.	Description	Contents
EVE HT	A High-throughput multi-cell counter	Main device 1 ea Desktop & monitor 1 set Multi-pipette 1 ea
EVH-020	EVE™ HT Counting kit	960 tests / kit Counting plate (48 channels × 20 plates) Mix well plate (96 wells × 10 plates) Trypan blue solution (0.4%, 20 mL × 2 bottles) Reservoir (20 pcs)
EHPQ-001	EVE™ HT QC plate (optional)	Low level, 1 pc
EHPQ-002	EVE™ HT QC plate (optional)	Middle level, 1 pc
EHPQ-003	EVE™ HT QC plate (optional)	High level, 1 pc
EHB-001	EVE HT Test Beads (optional)	1 x 1 mL / Pack
EHPP-001	EVE™ HT Preparation plate (optional)	Preparation plate
EVE HT 21 CFR Part 11	EVE™ HT 21 CFR Part 11 software (optional)	21 CFR Part 11 software

Safety Precautions

Review and follow the safety instructions below:

- If water or other material enters the instrument, the adaptor, or power inle disconnect the power cord and contact a service person. For operatin environment, refer to Product Specifications.
- Do not touch the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltag available at your location.
- This instrument is air-cooled and its surfaces may become hot durin operation. When installing, leave a space of more than 10 cm (4 inches around the instrument and do not place any objects between the instrumer and walls.
- Do not install an instrument on a slant or a place prone to vibrations, whic induces the risk of malfunction or damage of the instrument.
- Never insert any objects into the air vents of the instrument as this can resu in electric shock, personal injury, and equipment damage.
- Plug the power cord firmly into the wall outlet and AC adapter.
- To avoid potential shock hazard, make sure that the power cord is properl grounded.
- Be sure to position the instrument such that it is easy to disconnect.
- Turn off an instrument before unplugging the power cord and/or moving th instrument.
- If an instrument is dropped or broken, disconnect the power cord and contac a service person. The warrant will be void in case of disassembly.
- Use only authorized accessories (adaptor, power cord, and USB drive).

WARNING

Class A equipment is intended for use in an industrial environment. In th documentation for the user, a statement shall be included drawin attention to the fact that there may be potential difficulties in ensurin electromagnetic compatibility in other environments, due to conducted a well as radiated disturbances.

Mesures de sécurité

Examiner et suivre les instructions en matière de sécurité ci-dessous:

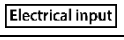
- Si de l'eau ou d'autres matières entrent dans l'instrument, l'adaptateur, o l'entrée de la prise, débrancher le cordon d'alimentation et contacter u technicien de service. Pour l'environnement d'exploitation, se reporter au Spécifications du Produit.
- Ne pas toucher la prise principale ou le cordon d'alimentation avec les main mouillées.
- S'assurer toujours que la tension d'alimentation correspond à la tensio disponible à votre localisation.
- Cet instrument est refroidi à l'air et ses surfaces peuvent devenir chaude pendant le fonctionnement. Lors de l'installation, laisser un espace de plu de 10 cm (4 pouces) autour de l'instrument et ne placer aucun objet entr l'instrument et les murs.
- Ne pas installer d'instrument sur une pente ou un endroit sujet aux vibrations qui entraînent un risque de défaillance ou de détérioration de l'instrument.
- Ne jamais insérer d'objets dans les évents d'air de l'instrument, car cela peu causer des chocs électriques, des blessures corporelles et des dommage de l'instrument.
- Mettre le cordon d'alimentation fermement dans la prise murale e l'adaptateur courant alternatif.
- Pour éviter tout risque de choc, s'assurer que le cordon d'alimentation es correctement mis à la terre.
- S'assurer de positionner l'instrument de telle sorte qu'il soit facile débrancher.
- Éteigner l'instrument avant de débrancher le cordon d'alimentation et/ou d le déplacer.
- En cas de chute ou de rupture d'un instrument, débrancher le cordo d'alimentation et contacter un technicien de service. La garantie sera annulé en cas de démontage.
- Utiliser uniquement les accessoires autorisés (adaptateur, cordo d'alimentation et clé USB).

AVERTISSEMENT

L'équipement de classe A est destiné à être utilisé dans un environnement industriel. Dans la documentation pour l'utilisateur, une déclaration doit être incluse pour attirer l'attention sur le fait qu'il peut y avoir des difficultés à assurer la compatibilité électromagnétique dans d'autres environnements, en raison de perturbations aussi bien conduisées que radiées.

Explanation of Safety Symbols

The following symbols are found on the medical device and this document. Always use the instrument in the safest possible manner.

Symbol	Meaning
	Caution & Warning
	Protective earth (Ground)
	Power On/Off
	The moving parts symbol indicates areas of the medical device in which moving parts can cause injuries. Do not operate the medical device with the door open.
	This equipment has been tested and found to comply with the limits for a Class A digital medical device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.
	This medical device and consumables conforms to the EC Declaration of Conformity.
	USB Connection
	This product conforms to UL 61010-1, CAN/CSA C22.2 No.61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements.” This instrument bearing the TÜV symbol are certified by TÜV Product Services to be in conformance with the applicable safety standard for the US and Canada.
	Catalogue number/Reference number
	Serial number
	Manufacturer
	Electrical input
	Consult Instructions for Use An electronic instructions for us (eLFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.

	Consult Instructions for Use An electronic instructions for us (eLFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.
	Disposal of your old appliance 1. When this crossed-out wheeled bin symbol is attached to a product it means the product is covered by the European Directive 2012/19/EU. 2. All electrical and electronic products should be disposed of separately from the municipal waste stream via designated collection facilities appointed by the government or the local authorities. 3. The correct disposal of your old appliance will help prevent potential negative consequences for the environment and human health. 4. For more detailed information about disposal of your old appliance, please contact local distributor, waste disposal service or call the number listed in the manual.
	LED
	Physician. Keep dry Keep away from rain
	Fragile, handle with care
	This way up
	General symbol for recover/recyclable
	Team lift
US Corporation	US Corporation
European Corporation	European Corporation
EC REP	Authorized representative in the European community
UK Representative	Authorized representative in United Kingdom
CH REP	Authorized representative in Switzerland
BRH	Authorized representative in Brazil

Warnings

1. After using this medical device, please turn off the main power.
If not, it may cause malfunction or lifetime reduction of medical device.

2. When turning off the medical device, make sure to lock the device by pressing 'Lock' button. If not, it may cause mechanical problem(s) or error message will appear during the device booting.

Item	Warning
Cover	Do not remove cover or disassemble case. There are no adjustable components inside the device. If malfunction occurs, please contact an authorized service person.
Manual	Do not attempt to service the device. This manual is only available in English. Failure to heed warnings may result in injury to service provider or operator.
Sample handling	Wear personal protective instrument during sampling and testing. Sample may contain infectious or bio-hazardous agents. Use of capped tubes and lint free wipes. Used lint free wipes shall be discarded.
Waste	After using the plate, dispose appropriately as bio-hazards waste. Do not reuse the plate.

Technical Support

Visit our Website at **www.nanoentek.com** for:

- Technical resources, including manuals, FAQs, etc.
- Technical support contact information
- Additional product information and special offers

For more information or technical assistance, please call or email.

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EVE™ HT

NESMU-EVEHT-001E (V.0.4)

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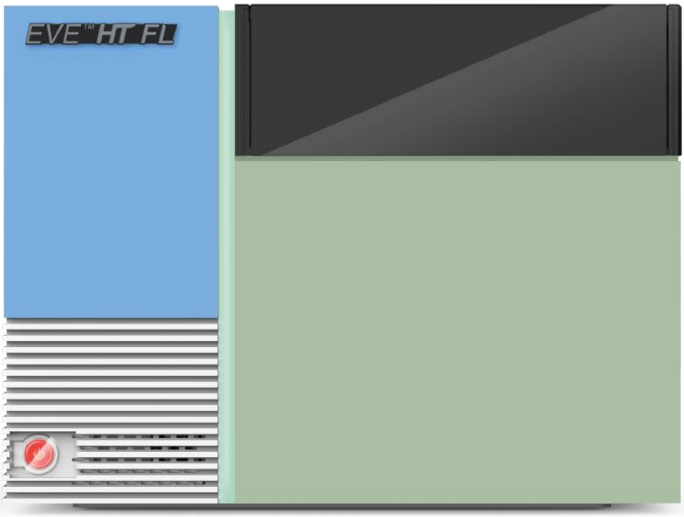
CE

EVE™ HT FL

User Manual



NanoEntek

EVE™ HT FL User Manual

Website: www.nanoentek.com

E-mail: sales@nanoentek.com

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The information in this user manual is described as accurately as possible.

Firmware and software changes may occur without prior notification.

Document number: **NESMU-EHTFL-001E (V.0.3)**

Revision history: **V.0.0 NOV 2023**
V.0.1 APR 2024
V.0.2 MAY 2025
V.0.3 SEP 2025

Table of Contents

Introduction

EVE™ HT FL is a high-throughput automated fluorescence cell counter. EVE™ HT FL can measure up to 48 samples simultaneously using disposable EVE™ HT FL Counting Plates. It takes only 3 minutes to measure all 48 samples with fast mode, and up to 20 minutes with accuracy mode. EVE™ HT FL requires 20 µL of samples to run measurements. EVE™ HT FL takes 2 fluorescence images (AO and DAPI channels) and optional bright field (BF) images. With BF images, EVE™ HT FL provides more accurate cell size histograms.

EVE™ HT FL can measure cell lines, primary cells, stem cells, and PBMCs. EVE™ HT FL has low user-to-user and instrument-to-instrument variations. EVE™ HT FL offers an optional “21 CFR part 11” module for data security and integrity which is compliant to FDA requirements.

Product Components

EVE™ HT FL consists of the following components.

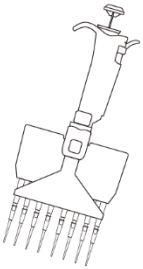
If any of the components are missing or damaged, please contact your local sales representative or send an email to **sales@nanoentek.com**.

EVE™ HT FL Instrument

1 EA

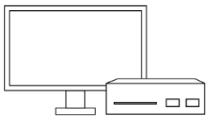
Multi pipette

1 EA



EVE™ HT FL desktop PC

1 SET



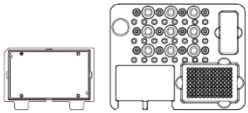
EVE™ HT FL counting kit

1 EA

EVE HT FL COUNTING KIT

EVE™ HT FL Accessories

Prepration plate (Optional)
QC plate (Optional)

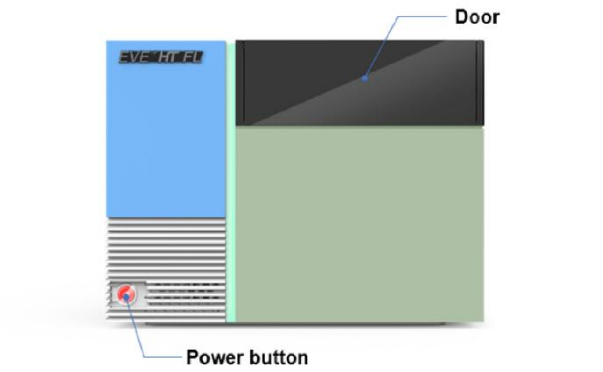


QC PLATE PREPARATION PLATE

EVE™ HT FL 21 CFR part 11 software (Optional)

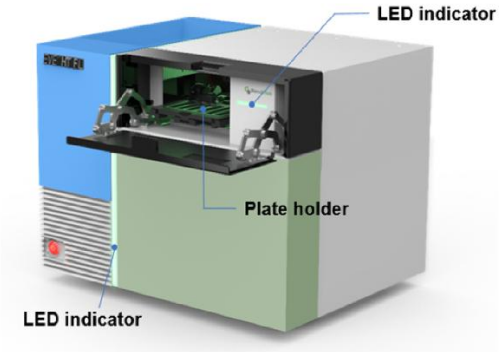
Product Description

Front view



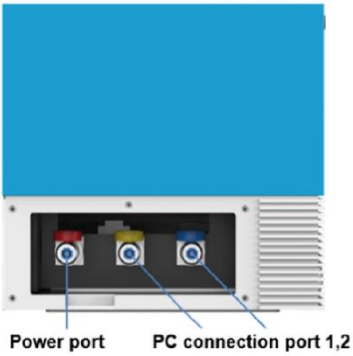
Part name	Description
Power button	Button to turn the instrument on or off
Door	Door to insert or retrieve EVE™ HT FL counting plate

Upper side view



Part name	Description
LED indicator	LED to indicate the state of EVE™ HT FL instrument
Plate holder	Holder to grip EVE™ HT FL counting plate tightly during measurements

Left side view



Part name	Description
Power port	Red port to supply power to EVE™ HT FL
PC connection ports	Yellow camera port and Blue port to communicate with EVE™ HT FL desktop PC

Installation

Environmental Requirements

For best performance, please see review the following recommendations to set up EVE™ HT FL:

- Use at room temperature.
- Do not expose instrument to direct sunlight.
- Do not expose instrument to continuous vibration.
- Do not expose instrument to intense magnetic or electromagnetic fields.
- Do not install instrument in high-humidity environment.
- Do not install instrument near corrosive gases or substances.
- Minimize contact with dust or airborne particles.
- Make sure to have at least 10 cm (4 inches) of free space around instrument for proper airflow.
- Avoid sharing an outlet and if possible, designate a wall outlet for EVE™ HT FL.

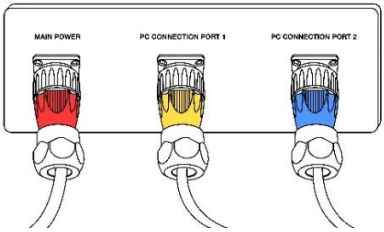
⚠ ***CAUTION***

If operating temperature is below 10 °C, wait for at least 10 minutes after turning on the instrument before use.

Installation

Installation and turning power on

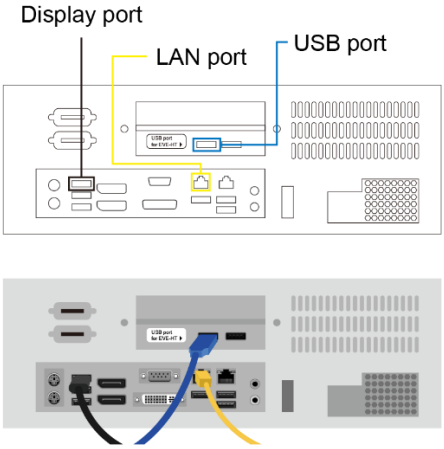
1. Find a flat space on a bench.
2. Open packages, put EVE™ HT FL instrument and EVE™ HT FL desktop PC on a flat space. Remove protective films.
3. Set up EVE™ HT FL desktop PC and monitor.
4. Unscrew 5 black bolts on the left side of EVE™ HT FL instrument to remove cover of side ports.
5. Connect three color coded connectors to matching ports.



6. Connect black cable to a wall outlet.
7. Connect yellow cable to the Ethernet port on the back of EVE™ HT FL desktop PC.

⚠ ***CAUTION***

- ***Make sure to connect yellow cable to the Ethernet port on a PCI board which is used for camera connection.***
- ***DO not connect yellow cable to the Ethernet port on the mother board which is used for internet connection***
- ***Please see the cartoon below.***



Installation

Installation and turning power on

8. Connect blue cable to a USB port on the back of EVE™ HT FL desktop PC.

⚠ ***CAUTION***

- ***Make sure to connect blue cable to one of the USB ports on a PCI board.***
- ***Do not connect blue cable to one of USB ports on the mother board.***

⚠ ***CAUTION***

- ***Do not tilt instrument too much when connecting the power cord.***
- ***Do not move instrument after connected to the power cord.***

9. Turn on EVE™ HT FL instrument and desktop PC.
10. Run EVE™ HT FL software.

⚠ ***CAUTION***

If an error code occurs during initialization, turn off both instrument and PC, and then restart both instrument and PC. If same error message appears repeatedly, contact your local distributor or sales@nanoentek.com.

Sample preparation

Recommended Actions

To obtain best results, follow these recommendations:

1. Handling sample

① Wear personal protective equipment while handling samples.
② Make sure to mix sample well at every step.
③ After loading prepared sample onto counting plate, wait for **2 minutes** to let cells settle down on the bottom surface.

2. Staining reagent

① Store the staining reagent at the appropriate temperature.
- AO/DAPI staining solution - in a refrigerator or on ice
- Trypan blue or Erythrosin B stain - at room temperature

3. EVE™ HT FL Counting Plate

① Keep counting plates in protective carriage case until use.
② Make sure to put counting plates on a clean surface.
③ Do not touch any other parts of counting plates except for the handles.
④ Make sure to fill entire well.
⑤ Do not tilt counting plate after loading samples.
⑥ Do not insert counting plate upside-down.
⑦ Make sure to push the plate all the way in.
⑧ Do not reuse those wells that have been filled up with samples. However, same plate can be used multiple times as long as there are unused wells.

4. Starting EVE™ HT FL software

① Make sure to turn on EVE™ HT FL instrument BEFORE starting EVE™ HT FL software.
② Make sure that door and plate holder are closed when starting EVE™ HT FL software.

Sample preparation

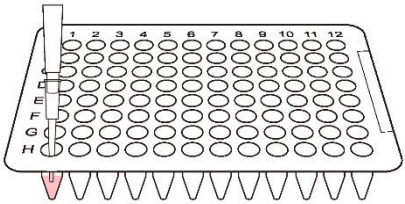
Staining samples

1. Prepare samples in growth media or PBS. Make sure that cells are well separated and suspended. If needed, vortex samples before starting staining procedure.

☛ ***NOTE***

EVE™ HT FL can analyze cell concentrations of 1 x 10^4 to 2 x 10^7 cells/mL.

2. If needed, concentrate or dilute samples.
3. Load **20 µL of well-mixed sample** into a mix well plate. ← Mix well plates (PCR plates) are included in EVE™ HT FL kit.

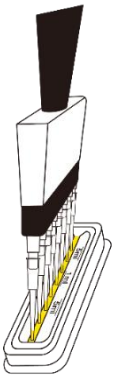


4. Dispense enough amount of **staining solution** to a reservoir. ← Disposable reservoirs are included in EVE™ HT FL kit.

☛ ***NOTE***

Use dispensed staining solution as soon as possible. It is not recommended to leave solution in a reservoir.

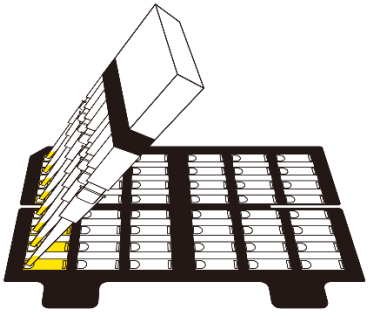
5. Add **20 µL of staining solution** to those wells that are loaded with cell samples using multi pipette. **Mix sample and staining solution well by pipetting up and down**. ← 8 channel pipette is included in EVE™ HT FL.



Sample preparation

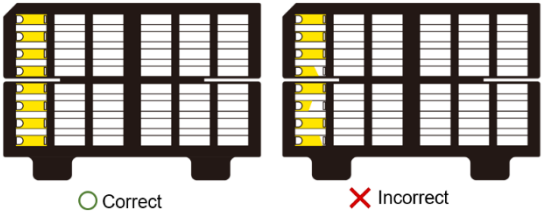
Staining samples

6. Load **20 µL of mixed samples** into EVE™ HT FL Counting plate.



☛ ***NOTE***

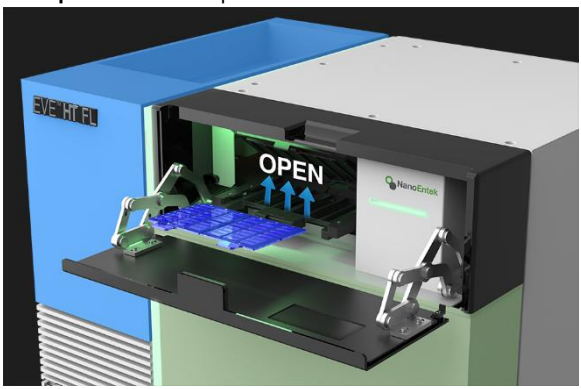
Examples of correctly loaded counting plate and incorrectly loaded counting plate



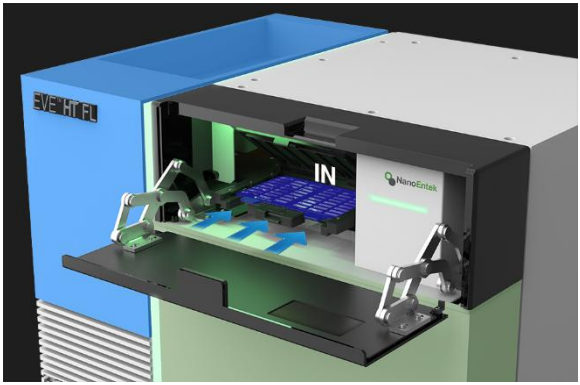
Sample preparation

Insert counting plate

1. Open black door in front of the instrument by pulling the door forward and find the black plate holder. Press the bar in the middle of the **plate holder** to open the holder.



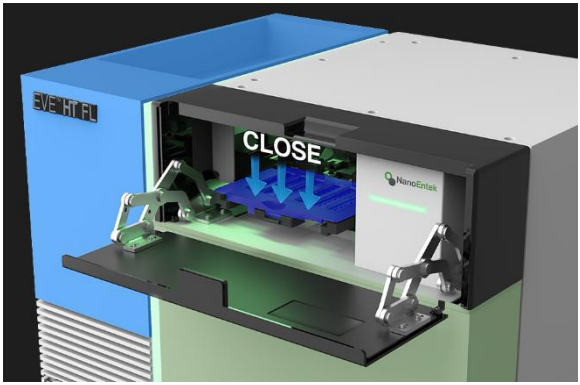
2. Insert **EVE™ HT FL Counting plate** loaded with samples into the plate holder.



Sample preparation

Insert counting plate

3. Close the **plate holder** by pressing the holder cap down until you hear “click” sound, and close the **door**.



⚠ ***CAUTION***

Allow samples to settle for ‘2 minutes’ after loading samples onto EVE™ HT FL Counting Plate. One can wait either before or after inserting plate.

⚠ ***CAUTION***

Make sure to put EVE™ HT FL Counting Plate all the way in.

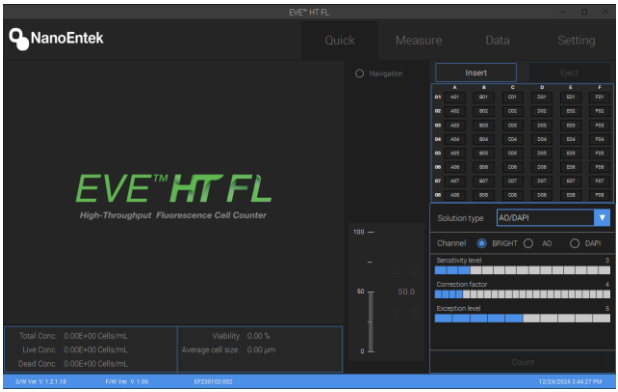
⚠ ***CAUTION***

Make sure to properly close plate holder cap and door before starting measurement.

Quick count

Quick count

The quick count function gives a quick measurement of what is being shown on live feed. One can use quick count function to determine whether sample needs further dilution or to adjust counting parameters.



1. Select the solution type.
2. To move the plate holder to imaging position, click the **'Insert'** button.
3. After counting plate is at imaging position, choose one of the wells loaded with samples.
4. Adjust focusing using up or down arrows. Please refer to example images below.

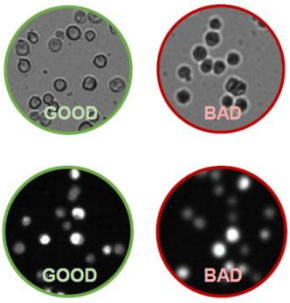
If there are more than one cell type, users will be asked to adjust focusing for each cell type. **Fine focusing** is also available. To use fine focusing, move mouse cursor on the preview window and turn mouse wheel while holding "ctrl" or "shift" key.

Quick count

Quick count

☛ ***Note:*** *Focus example*

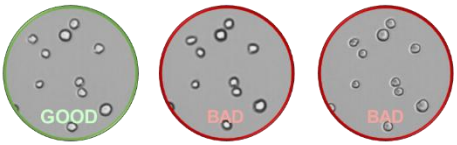
AO/DAPI



BRIGHT : The outline is clear, and the brightness inside the cells is the same as the background.

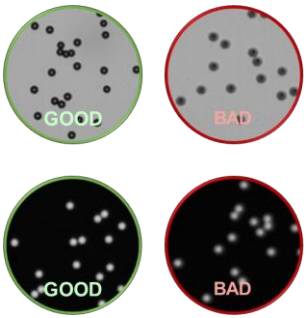
AO&DAPI : clear cell outline

Trypan blue or Erythrosin B



The outline is clear, and the brightness inside of live cells is brighter than the background.

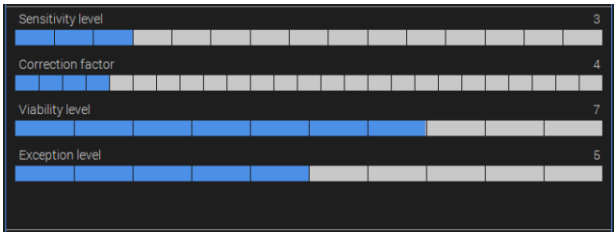
Test beads



The outline is clear in both the Bright and AO channels, with no blurriness.

Counting parameters

Counting parameters are used to deal with wide varieties of cell sizes and shapes. The description of each parameter is as follows.



1. BRIGHT channel

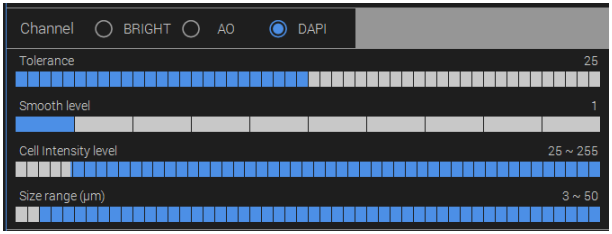
Sensitivity level : Decreasing this sensitivity parameter will make it easier to prevent debris from counting. (Caution: if the sensitivity level is too low, then some of the real cells may not be counted)

Correction factor : Decreasing this correction factor will make it easier to detect cells which are slightly darker than the background. When cells look very transparent, you can decrease this correction factor to pick up vague objects.

Viability level : Increasing this viability level will make it easier to detect marginally dark cells as dead cells. As you have tried already, increasing this level will increase the dead cell count.

☛ ***Note:*** *Viability level is available when using Trypan blue or Erythrosin B*

Exception level : Decreasing this exception level will make it easier to detect small objects.



2. AO or DAPI channel

(parameters need to be set for each channel)

Tolerance: Tolerance defines how to count dividing cells. Decreasing this parameter will allow counting cells in the middle of cell division or adjacent cells as individual cells. While increasing it will make large cells or aggregated cells to be counted as one cell.

Counting parameters

Smooth level: The smoothness level controls the smoothness of images. When cells are large and intracellular organelles are distinct enough to be counted as individual cells, you can increase this level to smooth out such distinctive textures.
Cell intensity level: Cell intensity level works similar to intensity gating. When cells are very dim or too bright, you can change the range of intensity levels of cells to be counted. Here, you can choose both minimum and maximum values.
Size range: Size range refers to the range of fluorescent cell sizes. This allows you to determine the size range and exclude small debris from the count.

To quickly test selected parameter sets, click the **'Count'** button and find results.

The following table provides examples of recommended parameters for commonly used cell lines. These are recommendations and can be further adjusted by users.

☛ ***Note:*** *Counting PBMCs or small diameter cells with Trypan blue or Erythrosin B is not recommended.*

☛ ***Note:*** *Using AO/DAPI solution is recommended for accurate cell count and viability analysis.*

AO/DAPI staining solution

	Cell size	Bright		
		Sensitivity level	Correction factor	Exception level
PBMC	2-80	3	4	5
CHO	5-80	3	4	5
Jurkat	5-80	3	4	5
U2OS	7-80	3	4	5
HeLa	8-80	3	4	5
HepG2	9-80	3	4	5

	AO				DAPI			
	Tolerance	Smooth level	Cell Intensity level	Size range	Tolerance	Smooth level	Cell Intensity level	Size range
PBMC	5	1	25-255	2-50	5	1	25-255	2-50
CHO	25	3	25-255	3-50	25	3	25-255	3-50
Jurkat	5	1	25-255	3-50	5	1	25-255	3-50
U2OS	5	1	25-255	3-50	5	1	25-255	3-50
HeLa	25	1	25-255	3-50	25	1	25-255	3-50
HepG2	25	3	25-255	3-50	25	3	25-255	3-50

Quick count

Counting parameters

Trypan blue stain

	Cell size	Sensitivity level	Correction factor	Viability level	Exception level
CHO	5-80	3	4	7	5
U2OS	7-80	3	4	7	5
HeLa	8-80	3	4	7	5

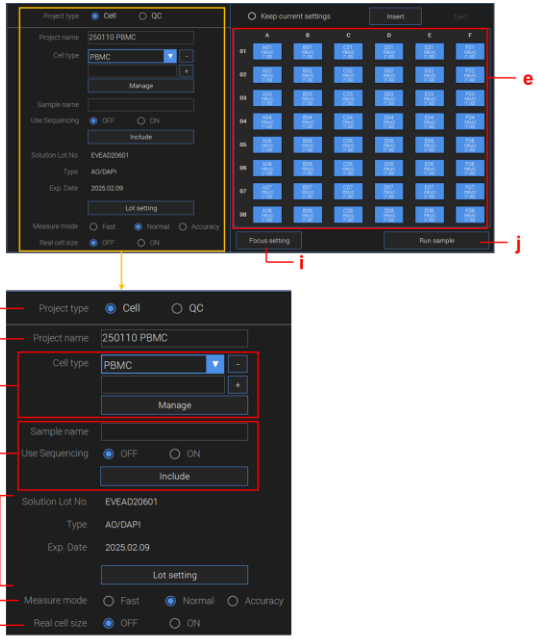
Erythrosin B stain

	Cell size	Sensitivity level	Correction factor	Viability level	Exception level
CHO	5-80	3	4	5	5
U2OS	7-80	3	4	5	5
HeLa	8-80	3	4	5	5

Measure

Measure setting

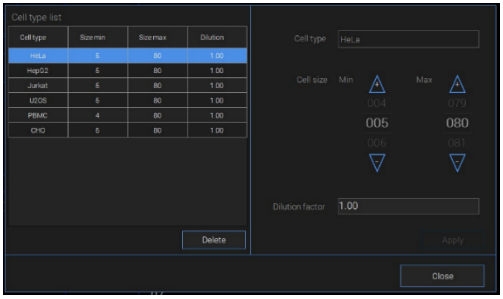
In the **Measure** tab, follow the steps described below to run measurements.



a. **Project type**: select “Cell” to run cell samples.
Select “QC” to run quality control samples. Please refer to ‘**QC**’ for details.

b. **Project name**: enter a project name.

c. **Cell type**: select one of the existing cell types or add a new one. Click **‘Manage’** to review and revise the preset size parameters.



Measure setting

d. **Define plate map**: repeat the following steps for all the wells to be measured.

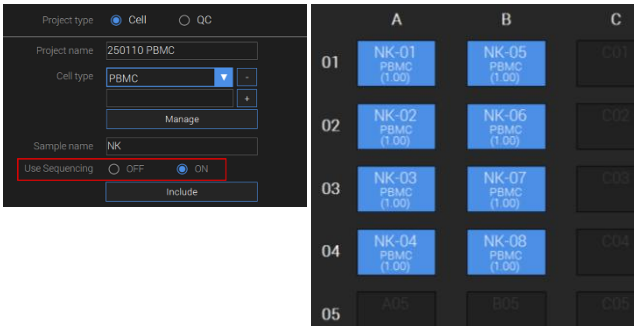
① After selecting a cell type, enter sample name. This field can be left blank.

② Select all the wells having this cell type in the plate map (**e**).

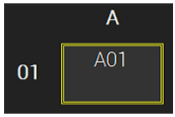
③ Click **'Include'** button.

If samples are replicates, one can group wells together by placing mouse cursor in the plate map, clicking the right mouse button and choosing "Include as a Group".

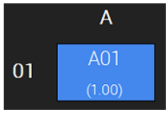
After entering the sample name, set **'Use Sequencing'** to **'ON',** and click **'Include'**, a sequential number is assigned to the sample name.



e. **Plate map**: click individual wells or click-and-drag group of wells to select wells.

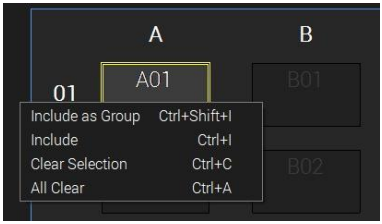


Selected well
(not included yet)



Included well

If you right-click on the well section, you can use functions such as Include and Exclude.



Measure

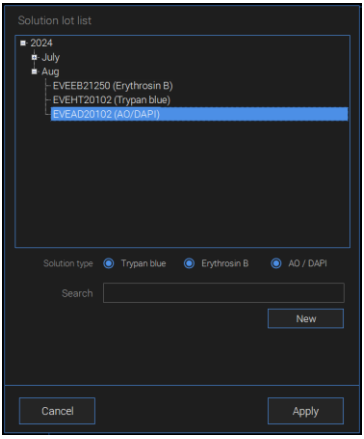
Measure setting

If you right-click outside the well, you can import or export plate maps.

If you select Format, you can download an Excel file with a blank format. You can enter information such as sample name and cell type directly into this Excel file before running measurements and then import predefined format.

☛ ***Note:*** *If the parameters in the Excel file do not match the values of the* ***Cell type****, the values from the Excel file will take priority, and the* ***Cell type*** *parameters will be updated accordingly.*

f. **Solution**: one can confirm the information about reagent solutions.
Click “Lot setting” to change or add new solution.



g. **Measure Mode**: choose “**Fast**” to take 1 image. If it is “**Normal**”, 4 images per well will be taken. A total of 15 images are taken if “**Accuracy**” is chosen.

☛ ***Note:*** *Only taking 4 images per well is available when using Trypan blue or Erythrosin B.*

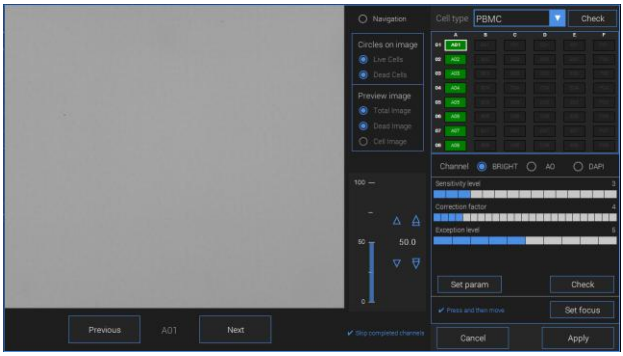
h. **Real Cell Size**: choose “**ON**” to take BR images which will give cell size estimates. (Available only when using AO/DAPI solution.)

i. **Focus setting**: see next page for details.

j. **Run sample**: click this button to start measurements.

Measure

Focus setting



When you press the focus setting button, the window below will appear. You can fine-tune the focus and parameters for each cell type here.

Navigation	Navigation on/off.
Circles	Circle (Live Cells / Dead Cells) on/off.
Preview image	Image (Total / Dead / Cell) on/off. *- Available only when using AO/DAPI solution.*
Previous/Next	Previous/Next move buttons.
Cell type	Select the cell type to set focus on and check it.
Channel	Select a channel to preview (BRIGHT / AO / DAPI) *- Available only when using AO/DAPI solution.*
Set param	Set current parameters.
Check	Count the cells on the current screen using the Quick count method.
Set focus	Apply the focus value.
Press and then move	After applying the focus setting, move to the next channel.
Skip completed channels	Channels that have been focused will be skipped.
Cancel/Apply	Cancel or apply current focus settings.

Measure

Focus setting

Follow the steps below to set focus.

1. Preview
- Zoom In and Out: put mouse cursor on the preview and turn mouse wheel.
- Select channel: Choose between BRIGHT, AO, or DAPI.

2. Plate map: Wells marked with BLUE indicate the wells of which focuses have not been set. When focuses are set, wells will be marked GREEN.
- Only the focus of the first well of each cell type needs to be adjusted.

3. Adjust focus: There are multiple ways to adjust focuses.
① Click single (fine steps) or double (coarse steps) arrows.
② Put mouse cursor on the preview, hold “ctrl” or “shift” key and turn mouse wheel.
Examples of good and bad focuses can be found on page 18.
③ Click **‘Check’** to see counting results of the current view.

4. Save focus: Click **‘Set focus’** to save focuses.
- If there are more than 1 cell type, preview will automatically move to the first well of the next cell type.

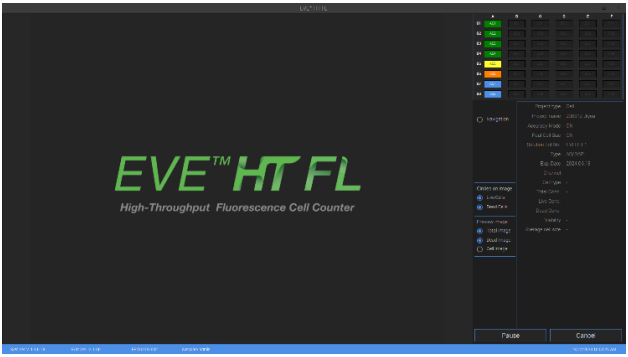
5. Image analysis parameters: Determine parameters for each cell type. Please see pages 19-21 for details.

6. Set param: If a parameter has been changed, press this button to save.

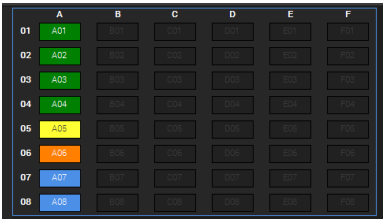
7. Finish focus setting: After saving focuses of all wells, click **‘Apply’** button.

Measurements and Calculations

After clicking **'Run sample'**, the following window appears, and the measurement will start following the plate map information entered previously.



You can check the current measurement status on the plate map in the upper right.



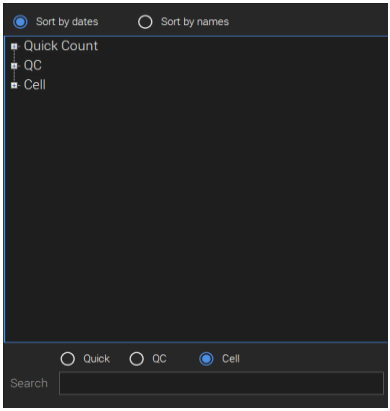
Blue: To be measured.
Orange: Image acquisition is in process.
Yellow: Image acquisition is done but image analysis is in process.
Green: Measurement has been completed.

If one clicks one of the GREEN wells, one can check images and results of the well.

Data

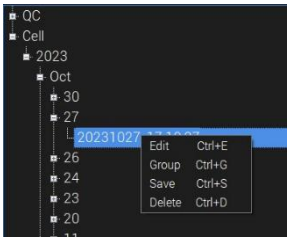
Data list

You can check the data list and results by clicking the **Data** tab.



View type	It can be viewed by two criteria, date or name.
Quick Count	Data list taken from the Quick menu.
QC	Data list taken from the QC mode (Project type).
Cell	Data list taken from the Cell mode (Project type).
Search	Search the data in each section.

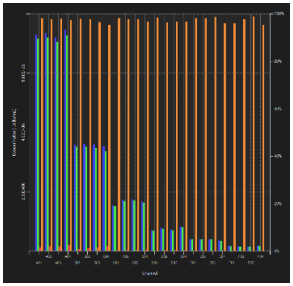
Right-click on each project to find sub-menu.

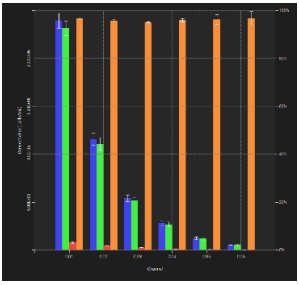


Edit	Rename the project name. Set the Size gating and dilution factor. Acceptance range is only available in QC data.
Group	To edit group setting, select **Group**.
Save	To save project, select **Save**. Select the data type and data path in the pop-up window.
Delete	To delete project, select **Delete**.

Graph and Table

Data measured with Quick Count, results will only include images and counting summary.
Data measured with QC as a project type, results will show whether results are consistent. (See Quality Control chapter for details.)
Data measured with Cell as a project type, results will appear in graph and table form.





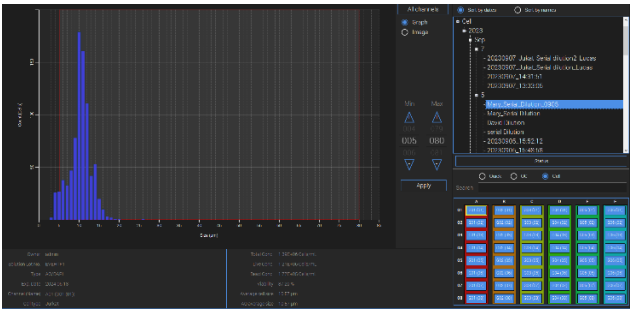
The graph shows total counts (BLUE), live cell counts (GREEN), dead cell counts (RED), and viabilities (ORANGE). You can hide any of these graphs by unchecking boxes below the graph. Grouped data will be displayed as a single graph.

Channel	Name	Total Conc.	Live Conc.	Dead Conc.	Viability (%)	Average Size (µm)
Group	G01	2.39E+06	2.31E+06	7.69E+04	96.79	11.36
	Std dev	7.64E+04	7.41E+04	8.47E+03	0.33	0.10
	Std err	3.82E+04	3.70E+04	4.24E+03	0.16	0.05
	CV	3.19	3.20	11.02	0.34	0.90
Group	G02	1.16E+06	1.11E+06	4.90E+04	95.75	11.37
	Std dev	6.31E+04	6.49E+04	6.67E+03	0.67	0.08
	Std err	3.15E+04	3.25E+04	3.33E+03	0.33	0.04
	CV	5.45	5.85	13.60	0.70	0.67
Group	G03	5.40E+05	5.14E+05	2.61E+04	95.17	11.30
	Std dev	3.64E+04	3.49E+04	1.69E+03	0.14	0.15
	Std err	1.82E+04	1.74E+04	8.46E+02	0.07	0.08
	CV	6.74	6.79	6.49	0.15	1.33
D05	D05	2.65E+05	2.56E+05	8.84E+03	96.67	11.55
D06	D06	2.97E+05	2.86E+05	1.06E+04	96.43	11.54
D07	D07	3.04E+05	2.92E+05	1.24E+04	95.93	11.42
D08	D08	2.46E+05	2.33E+05	1.24E+04	94.96	11.39
E05	E05	1.08E+05	1.02E+05	5.30E+03	95.08	11.42
E06	E06	1.24E+05	1.20E+05	3.53E+03	97.14	11.87
E07	E07	1.40E+05	1.38E+05	1.77E+03	98.73	11.47
E08	E08	1.22E+05	1.15E+05	7.07E+03	94.20	11.55
F05	F05	6,01E+04	5,83E+04	1,77E+03	97.06	10.94
F06	F06	6.01E+04	6.01E+04	0.00E+00	100.00	11.70
F07	F07	4.95E+04	4.59E+04	3.53E+03	92.86	11.10
F08	F08	5.83E+04	5.65E+04	1.77E+03	96.97	11.46

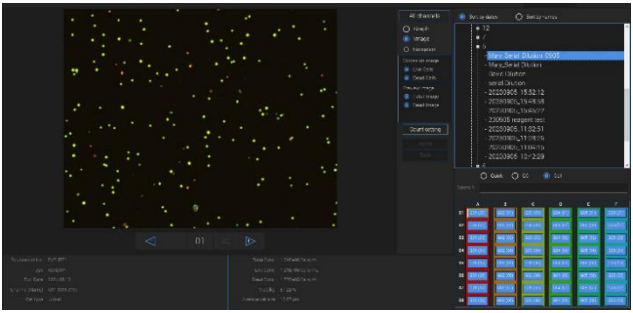
Data for each well (or group) is organized and displayed in a table as shown above. When you click on one of the column names, results will be sorted in ascending or descending order of the selected column (Group data cannot be sorted). For grouped data, the standard deviation, standard error, and CV of each group are automatically calculated and displayed.

Individual well results

When one well is selected, cell counts (Total, Live and Dead cell concentrations), viability, and average cell size will be shown below cell size histogram. You can change minimum or maximum cell sizes to be included in the results.

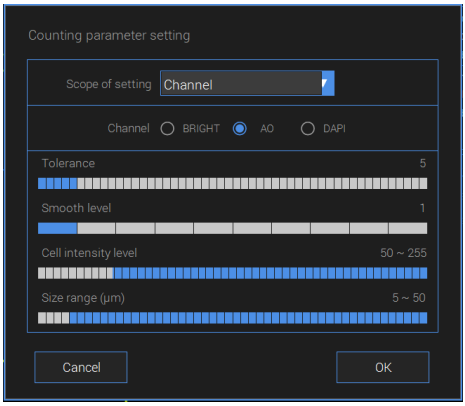


By selecting **'Image'**, you can review raw images. If **'Real cell size'** has been selected when using AO/DAPI solution, bright channel images will also be shown.



Individual well results

If you click **'count setting'**, you can change counting parameters in the current data. Refer to page 19-21 for a detailed explanation of parameters. Parameter values can be applied simultaneously to Channel, Cell type, Group, or Project.



By right-clicking and dragging the image, you can select cells individually as shown below to manually assign those objects in the selected area to either live or dead cells, or debris.

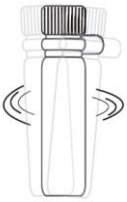
Quality control

QC bead preparation

Bead counting can be used as a way to evaluate whether instrument is in good condition.

☛ ***Note:*** *Ordering information is on page 60-61.*

1. Shake bottle vigorously or vortex briefly for 5 seconds before use.

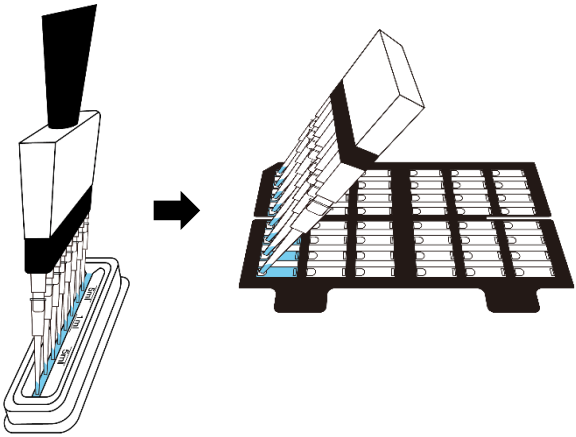


[Shake bottle vigorously]

[Vortexing]

2. Transfer **20 µL of test beads** to the Mix Well Plate.
For **FL test beads**, add **20 µL of PBS**.
For **BR test beads**, add **20 µL of Trypan blue or Erythrosin B**.
Mix well by pipetting up and down.

3. Take 20 µL of diluted test beads using a multi-pipette and load onto a counting plate.



QC bead Preparation

4. Open EVE™ HT FL door and open plate holder.

5. Insert counting plate loaded with sample into the plate holder.

6. Close the plate holder cap and close the door.

⚠ ***CAUTION***
Allow beads to settle for '1 minute' before starting measurements.

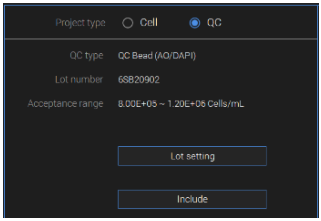
⚠ ***CAUTION***
Make sure to push the plate all the way in.

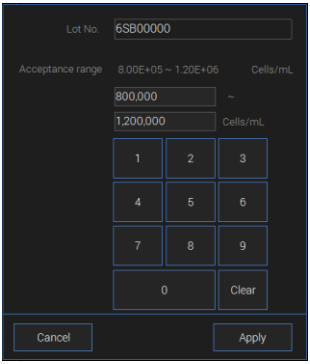
⚠ ***CAUTION***
Make sure door and plate holder are properly closed.

QC bead Run

After loading QC beads onto a counting plate, proceed with the settings in the Measure tab as follows.

1. Select **QC** as a project type and click '**Lot setting**' when using a new lot or for the first time.



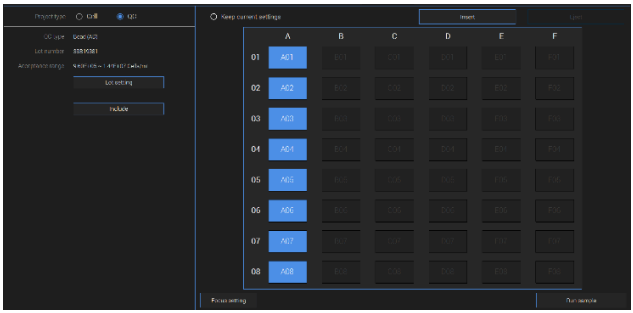


2. Click **'New'** button, enter lot number and acceptance range as written in the label of test beads, and then click **'Apply'** to save.

QC bead Run

3. Select the lot you use and click **‘Apply’** button.

4. Select the wells loaded with QC samples. For multiple selection, left click and drag.



5. Click **‘Include’** button or select ‘Include’ option from sub-menu by right clicking. Make sure selected wells are displayed in blue as shown.

6. Click **‘Focus Setting’** to adjust focus.

7. Adjust focuses on both BRIGHT and AO channels for FL test beads, or on BRIGHT only for BR test beads.

☛ ***Note:*** *Focusing for DAPI channel will be automatically updated based on AO focus position.*

☛ ***Note:*** *For information on how to focus, see page 18.*

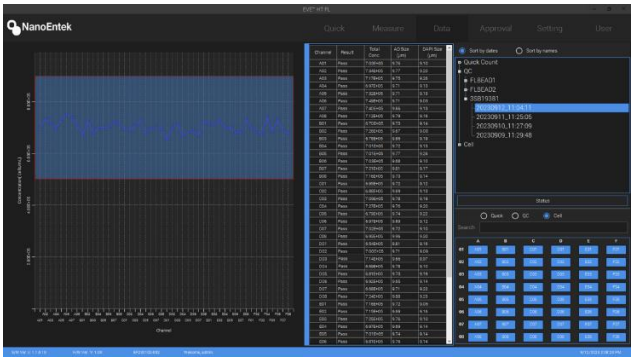
8. After focusing, click **‘Set focus’** button to save and then click **‘Apply’** button.

9. Click **‘Run sample’** button to start measurement.

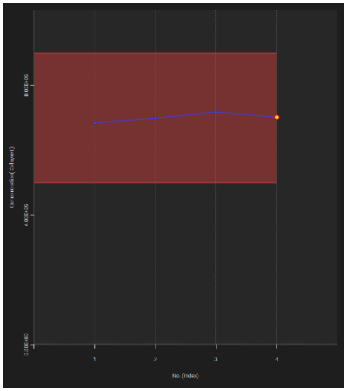
Quality control

QC bead Run

After measurement is completed, select data from the data list and check the dot graph as shown below. One can check total counts of beads, and sizes of beads.



If you click on a bead lot number in the data list, you can see the results of all previous measurements of the selected lot.



Quality control

QC plate Preparation

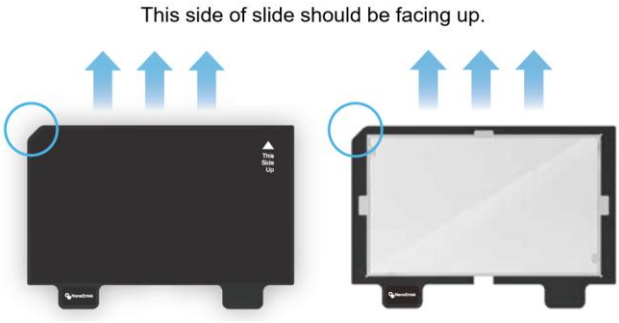
This is for quality control using QC plate. Follow the instruction below only if necessary.

1. Prepare the QC Plate.

☛ ***Note:*** *Ordering information is on page 60-61.*

2. Open the EVE™ HT FL door and open plate holder.

3. Insert the **QC Plate** into the plate holder.



4. Close the plate holder cap and close the door.

⚠ ***CAUTION***

Make sure to push the plate all the way in.

⚠ ***CAUTION***

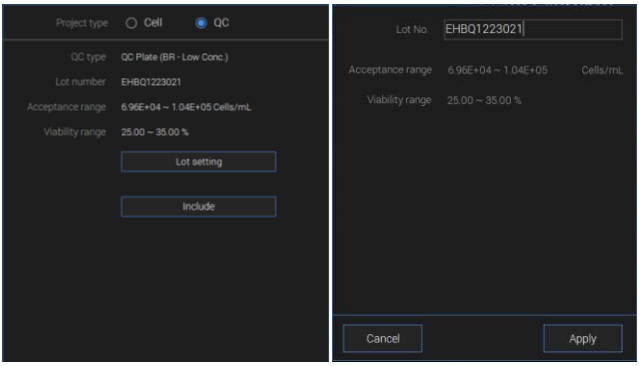
Make sure door and plate holder are properly closed.

Quality control

QC plate Run

After insert the QC plate into the instrument, proceed with the settings in the Measure tab as follows.

1. Select **QC** as a project type and click '**Lot setting**' when using a new lot or for the first time.



2. Click **'New'** button, enter lot number and check the acceptance range as written on the plastic package label of QC plate, and then click **'Apply'** to save.

3. Select the lot you use and click **'Apply'** button.

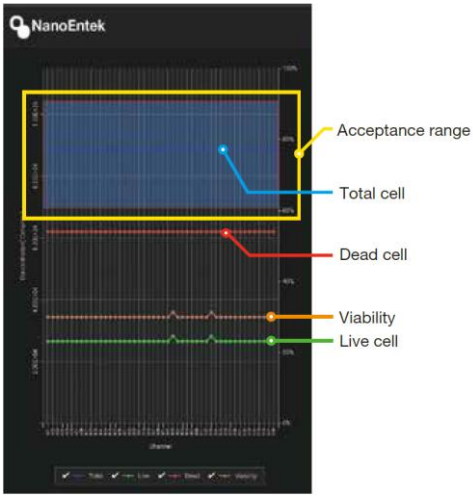
4. Select all 48 channels. For multiple selection, left-click and drag.

QC plate Run

5. Click **'Include'** button or select 'Include' option from sub-menu by right clicking. Make sure selected wells are displayed in blue as shown.

6. Click **'Run sample'** button to start measurement.

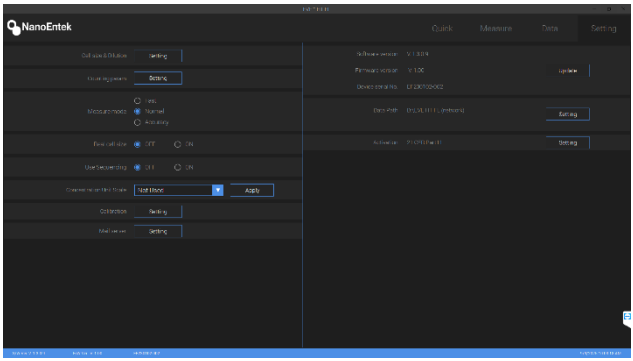
After measurement is completed, select data from the data list and check the dot graph as shown below.



If you click on a plate lot number in the data list, you can see the results of all previous measurements of the selected lot.

Setting

Setting tab



In **Setting**, you can set default values for parameters and dilution factors. These value sets will be used when a cell type is not specified or a new cell type is created.

Cell size & Dilution	Cell size and dilution factor of sample
Counting parameter	Parameter values for each BRIGHT, AO, and DAPI channel
Measure mode	Default analysis mode when using AO/DAPI staining solution
Real cell size	Default setting when using AO/DAPI staining solution
Use Sequencing	Default setting for assigning sequential number after the sample name
Concentration Unit Scale	Fixes the display of concentration values in exponential format (1.00E+01 to 1.00E+09). **Not applied to QC data*
Calibration	Calibration of brightness Calibration of the instrument image background level (Only for Trypan blue or Erythrosin B stain)
Mail server	**Do not change Mail settings.*
SW, HW information	Information about installed software and hardware
Data path	Path where measurement images and data are saved
21 CFR part 11 activation	Activate an optional program that complies with 21 CFR part 11

☛ ***Note**: Mix ratio (sample 20 µL + reagent 20 µL) is already applied so do not apply to dilution factor.*

Calibration

Backgrounds that are overly bright or dark may affect the results. Do the Background calibration when using Trypan blue or Erythrosin B with a new lot number.

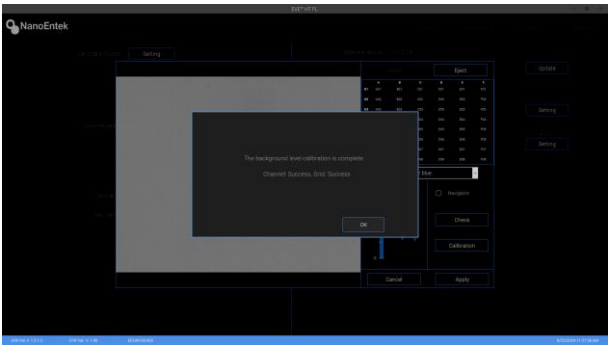
1. Calibration sample preparation
   - ① Mix **20 µL of culture media** and **20 µL of Trypan blue or Erythrosin B** thoroughly.
   - ② Load **20 µL of the mixture** into one channel in an EVE™ HT FL Counting Plate.
   - ③ Insert the counting plate into the plate holder of the instrument.

2. Calibration setting
   - ① Select '**Setting'** tab.
   - ② Click **Calibration 'Setting'** button.

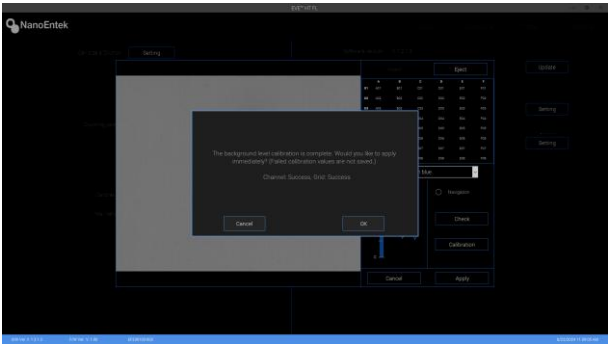
   - ③ Select the solution type for calibration
   - ④ Click **'Insert'** button.

Calibration

⑤ Select the wells loaded with the mixture.



⑥ Click **‘Check’** button.



⑦ Click **‘Calibration’** button.

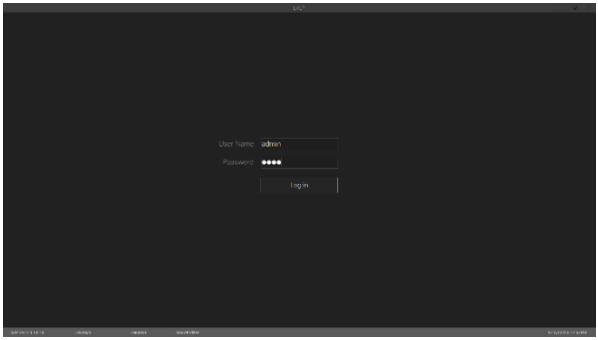
⑧ After finish the calibration, click **‘OK’** and **‘Apply’** button.

21 CFR part 11

The Food and Drug Administration (FDA) of the United States has established regulations on electronic records and electronic signatures (ERES) in Title 21 of the Code of Federal Regulations, specifically 21 CFR Part 11. EVE HT FL offers 21 CFR part 11 compliance program for cGMP facilities as an option. Please contact technical support to add this option.

Log In

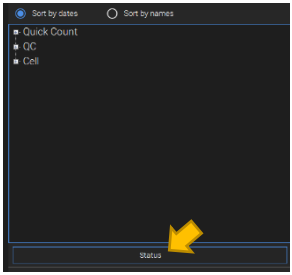
When activating 21 CFR Part 11, the user must log in on the first screen. Log in from login screen. (default ID/PW : admin/0000)

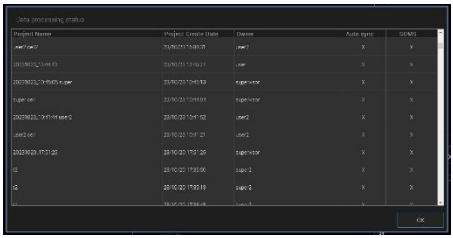


☛ ***Note**: Log out is in the User tab.*

Data status

Users can check data status upon 21 CFR Part 11 activation.

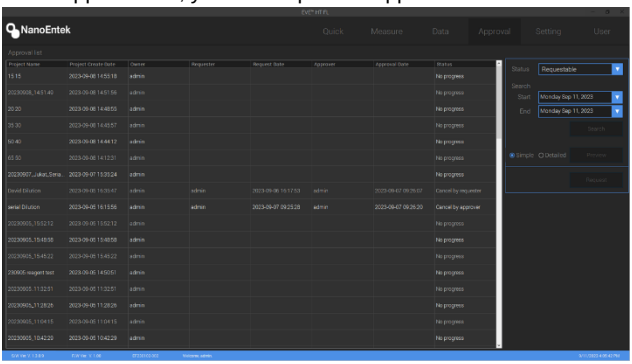




21 CFR part 11

Approval

In the approval tab, you can request or approve data.



You can check the report file for the data by double-clicking the data or clicking the Preview button.

Data list is divided into four status (Requestable, Requesting, Approvable, Approved) types depending on the approval status.

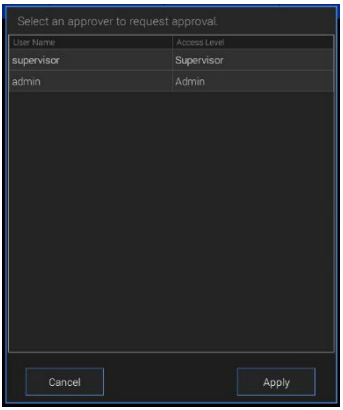
1. **Requestable**

The list shows data that can be requested for approval.

① Select the data to get approval.

② Click the **'Request'** button.

③ Enter the requester's password.



④ Select an approver to request approval.

⑤ Click the **'Apply'** button.

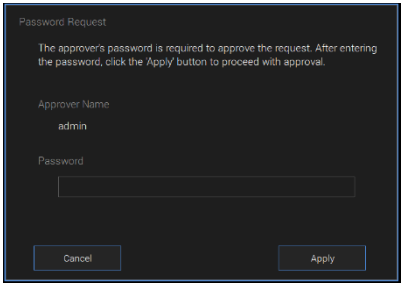
Approval

2. **Requesting**

The requesting data are displayed. It is possible to **'Cancel'** the request for approval. The approver can approve the request in Requesting tab. The approval no need to log in to the approval ID.

① Select the Data in list

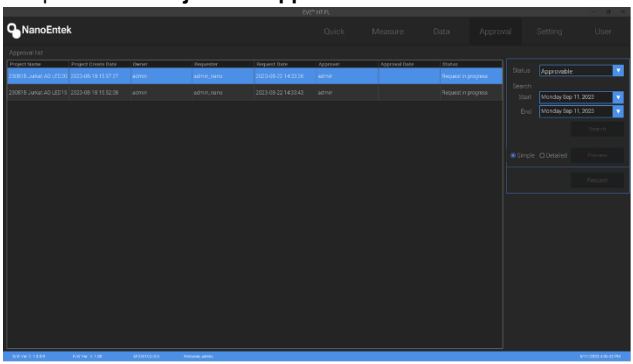
② Click the **'Approval'**



③ Enter the approver's password.

3. **Approvable**

The approvable data are displayed on this tab.
It is possible to **'Reject'** or **'Approval'**.



4. **Approved**

The approved data is listed.
It is possible to **'Export'** the Approved data.

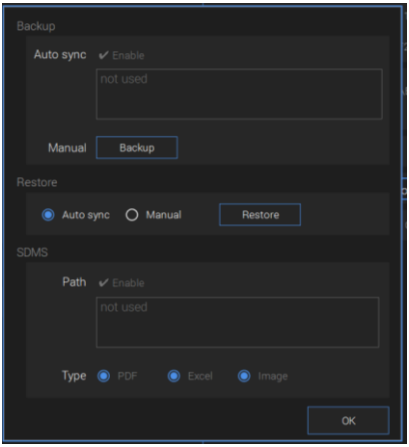
21 CFR part 11

Setting

In the 21 CFR part 11 program, several functions are added to the setting tab.

1. Backup & Restore

① Click the Backup & Restore **'Setting'** button.



② To enable automatic backup, click the Auto sync **'Enable'**, set the backup data path.

③ Click the Manual **'Backup'** button, save the backup data at the current point in time.

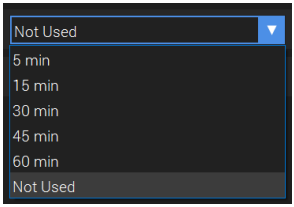
④ The Restore function provides two options. You can back up based on the backup data saved by Auto sync or the backup data saved by Manual.

⑤ Click the SDMS Path **'Enable'**, set the SDMS data path.

⑥ Select the desired type of SDMS data.

2. Auto logout

① Click the '▽' button.



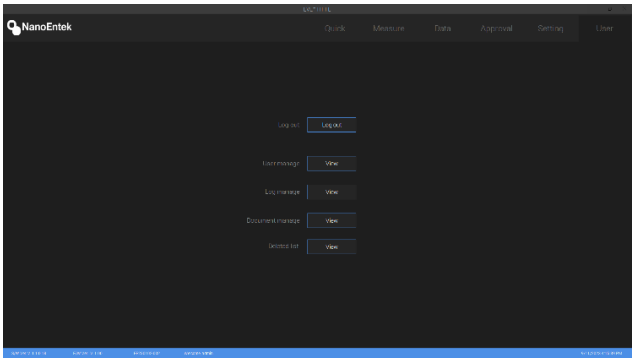
② Select the auto logout limit time.

③ Click the Auto Logout **'Setting'** button.

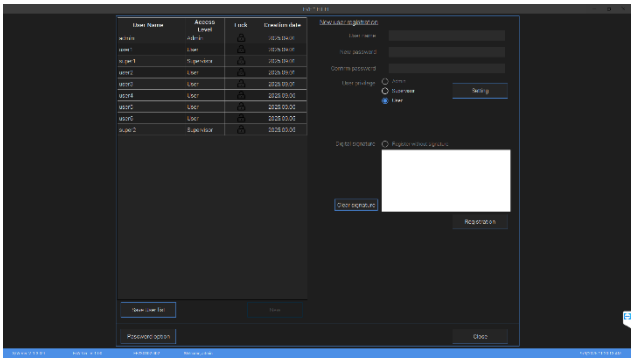
21 CFR part 11

User manage

When the 21 CFR part 11 program is activated, the user tab becomes available.



Click the User manage **'View'** button.



1. New user registration.

① Enter the user ID and PW.

② Click the User privilege **'Setting'**.

③ Set the User privilege in each menu.

④ Set the User and Supervisor permission.

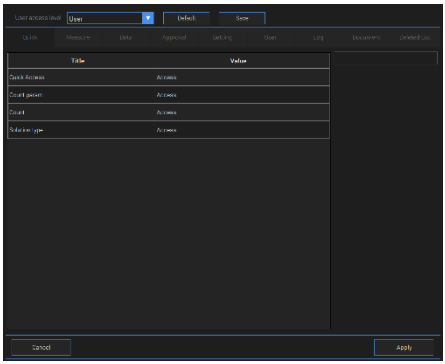
⑤ Click the **'Apply'** button.

⑥ Enter the signature and Click the **'Registration'.**

☞ ***Note**: **'Registration without signature'** is allowed, and the user will be required to register their signature at the time of first login.*

21 CFR part 11

User manage



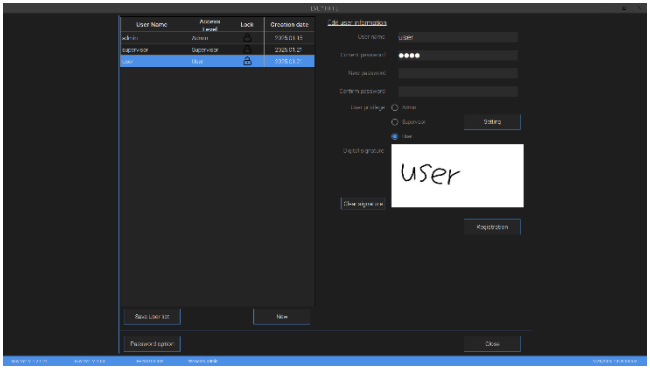
User and Supervisor default permission settings can be changed.
- Set the privilege and click the **‘save’** button.
- See the 21 CFR part 11 Supplement for default settings.

2. Edit the user option

① Select the user in user list.

② Do the same process in Creating New user.

③ Depending on the user's granted privileges, the account can be locked.

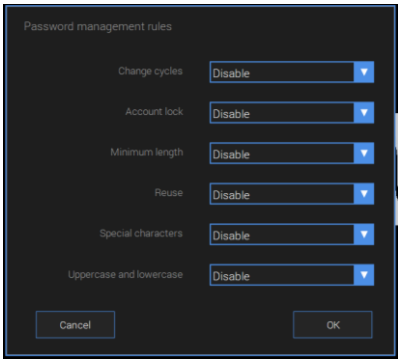


3. Password option

Set the password management rules.

21 CFR part 11

User manage



① Change cycles.
Disable, 30 days, 60 days, 180 days.
② Account lock
Disable, ≥ 3 times, ≥ 5 times, ≥ 10 times, ≥ 15 times.
③ Minimum length
Disable, ≥ 3, ≥ 5, ≥ 10, ≥ 15.
④ Reuse
Disable, ≥ 30 days, ≥ 60 days, ≥ 180 days.
⑤ Special characters
Disable, Enable.
⑥ Uppercase and lowercase
Disable, Enable.

4. Lock in user list

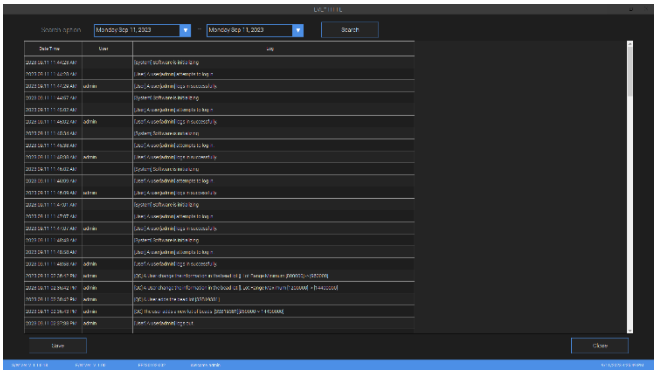
User Name	Access Level	Lock	Creation date
admin	Admin		2025.01.15
supervisor	Supervisor		2025.01.21
user	User		2025.01.21

User ID is locked when login fails. Lock icon turns red.
Click the button to unlock user ID and the button changes to grey.

21 CFR part 11

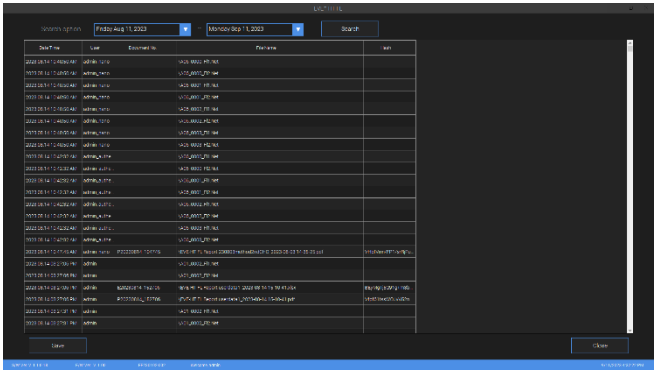
Log manage, Document manage, Deleted list

1. Log manage



This log manage allows you to view a list of instrument log.

2. Document manage

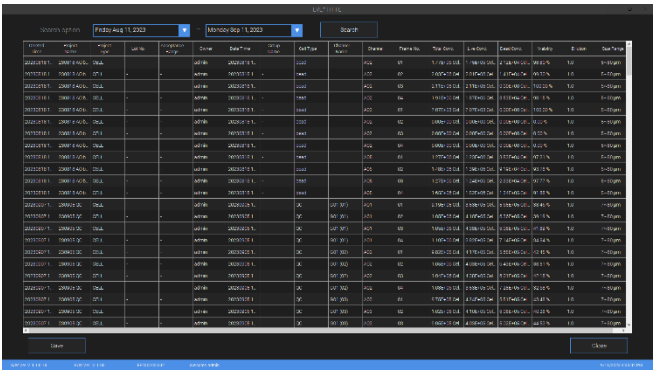


This document manage allows you to check the documents made in the equipment.

21 CFR part 11

Log manage, Document manage, Deleted list

3. Deleted list



This deleted list allows you to view a list of deleted data.

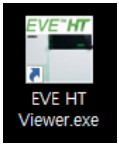
☛ ***Note**: EVE HT FL provides a comprehensive solution to comply with the requirements of 21 CFR Part 11. For more information on these features, please refer to the "Support for 21 CFR Part 11."*

Viewer program

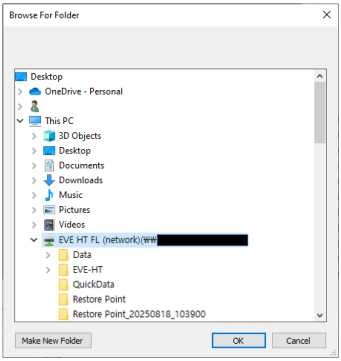
Viewer setup

EVE HT FL supports a viewer program that allows users to view, edit, and approve data on a local PC. The viewer program provides the same features as the main software, except for the functions in the Quick and Measure menus, including related settings.
Data can be accessed through a network drive connection with the EVE HT FL instrument. For this connection, the data folder must be configured for network sharing.

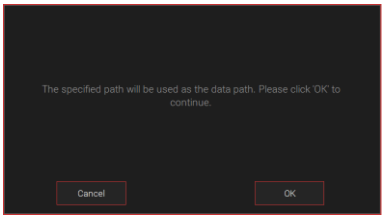
After installing the Viewer program on the local PC to be used, configure the initial settings according to the following guide:



1. Launch the EVE HT Viewer program. When the program is launched, a prompt window will appear asking you to specify the data path. Click OK to continue.



2. Specify the data path to the network folder.



3. A prompt window will appear indicating that the data path will be changed. Click OK to continue.

4. The Viewer interface will be displayed.

Maintenance and Cleaning

Clean the surface of EVE™ HT FL instrument with a damp cloth. If liquid spills on EVE™ HT FL, turn off the power immediately and wipe dry.

EVE™ HT FL does not need regular maintenance. To troubleshoot problems with EVE™ HT FL, contact technical support.

⚠ **IMPORTANT! Never disassemble or service EVE™ HT FL by yourself.**

Unauthorized repairs may damage EVE™ HT FL or alter its functionality, which will void your warranty. Contact **sales@nanoentek.com** or your local distributor to arrange for service.

⚠ **IMPORTANT! Always wipe surfaces with ethanol-soaked paper towels.**

Do not directly spray ethanol anywhere on EVE™ HT FL.

⚠ **IMPORTANT! Avoid exposing EVE™ HT FL to UV light.**

UV light may degrade components, including plastic.
Damage from UV exposure is not covered under the manufacturer's warranty.

Troubleshooting

Installation	
EVE™ HT FL does not power on	• Check on/off switch on left side of main instrument. • Check power source or contact your distributor.
Operator software does not start	• Check on/off switch on back side of main instrument. • Check connection between instrument and PC.
Inaccurate result	
Low and high results	• EVE™ HT FL is designed to read samples from 1×10^4 cells/mL to 2×10^7 cells/mL. • If your sample is out of this range, you may need to dilute the sample or add more cells and read the sample again.
Dilution factor	• Check the mixing ratio of Sample 20 µL + staining solution 20 µL. Mix ratio is already applied so do not apply to dilution factor. • Apply sample dilution to the dilution factor.
Dust or bubbles	• Check the surface of EVE™ HT FL Counting Plate. • Be careful not to make any bubbles when mixing and loading sample with a pipette. • Set the ‘Counting parameter’ before count in Focus setting window. Refer to page 19-21. • Remove any bubbles and dust in the image after count using the image edit function. Refer to page 31.
Incorrect focus	• Set the correct focus. Refer to page 18.
Too big or too many clumpy cells	• Ensure the cells are not clumped.
Inaccurate result in Bright field counting reagent	• If your sample contains small cells, Trypan Blue or Erythrosin B may not provide accurate result. Use AO/DAPI solution will improve the accuracy of the cell count. • For accurate viability determination, AO/DAPI solution is recommended.
Plate	• Push the plate all the way in.
Saving problems	
E-mail	• Check the internet connection.
USB storage	• Check the storage path.

Warranty

NanoEntek provides (1) year warranty service for defects of material and workmanship.

If any defects occur in EVE™ HT FL, NanoEntek provides repair services for the defective parts at its discretion.

The following defects, however, are specifically excluded:

1. Defects caused by improper operation.
2. Repair or modification done by anyone other than NanoEntek or an authorized agent.
3. Damage caused by substituting alternative parts.
4. Use of fittings or spare parts supplied by anyone other than NanoEntek.
5. Damage caused by accident or misuse.
6. Damage caused by disaster.
7. Corrosion caused by improper solvent or sample.

For your protection, EVE™ HT FL units being returned must be insured against possible damage or loss. NanoEntek cannot be responsible for damage incurred during shipment of a defective instrument. It is recommended that you save the original packing material in which the instrument was shipped. This warranty is limited to the replacement of defective products.

For any inquiry or request for repair service, please contact **sales@nanoentek.com** or your local distributor.

Safety precautions

Review and follow the safety instructions below:

- If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to Product Specifications.
- Do not touch the main plug or power cord with wet hands.
- Always ensure that the power supply input voltage matches the voltage available at your location.
- This instrument is air-cooled and its surfaces may become hot during operation. When installing, leave a space of more than 10 cm (4 inches) around the instrument and do not place any objects between the instrument and walls.
- Do not install an instrument on a slant or a place prone to vibrations, which induces the risk of malfunction or damage of the instrument.
- Never insert any objects into the air vents of the instrument as this can result in electric shock, personal injury, and equipment damage.
- Plug the power cord firmly into the wall outlet and AC adapter.
- To avoid potential shock hazard, make sure that the power cord is properly grounded.
- Be sure to position the instrument such that it is easy to disconnect.
- Turn off an instrument before unplugging the power cord and/or moving the instrument.
- If an instrument is dropped or broken, disconnect the power cord and contact a service person. The warranty will be void in case of disassembly.
- Use only authorized accessories (adaptor, power cord, and USB drive).

⚠ ***WARNING***

Class A equipment is intended for use in an industrial environment. In the documentation for the user, a statement shall be included drawing attention to the fact that there may be potential difficulties in ensuring electromagnetic compatibility in other environments, due to conducted as well as radiated disturbances.

Mesures de sécurité

Examiner et suivre les instructions en matière de sécurité ci-dessous:

- Si de l'eau ou d'autres matières entrent dans l'instrument, l'adaptateur, ou l‘entrée de la prise, débrancher le cordon d'alimentation et contacter un technicien de service. Pour l'environnement d'exploitation, se reporter aux Spécifications du Produit.
- Ne pas toucher la prise principale ou le cordon d'alimentation avec les mains mouillées.
- S‘assurer toujours que la tension d'alimentation correspond à la tension disponible à votre localisation.
- Cet instrument est refroidi à l'air et ses surfaces peuvent devenir chaudes pendant le fonctionnement. Lors de l'installation, laisser un espace de plus de 10 cm (4 pouces) autour de l'instrument et ne placer aucun objet entre l'instrument et les murs.
- Ne pas installer d'instrument sur une pente ou un endroit sujet aux vibrations, qui entraînent un risque de défaillance ou de détérioration de l'instrument.
- Ne jamais insérer d'objets dans les évents d'air de l'instrument, car cela peut causer des chocs électriques, des blessures corporelles et des dommages de l'instrument.
- Mettre le cordon d'alimentation fermement dans la prise murale et l'adaptateur courant alternatif.
- Pour éviter tout risque de choc, s‘assurer que le cordon d'alimentation est correctement mis à la terre.
- S‘ssurer de positionner l'instrument de telle sorte qu'il soit facile à débrancher.
- Éteigner l’instrument avant de débrancher le cordon d'alimentation et/ou de le déplacer.
- En cas de chute ou de rupture d'un instrument, débrancher le cordon d'alimentation et contacter un technicien de service. La garantie sera annulée en cas de démontage.
- Utiliser uniquement les accessoires autorisés (adaptateur, cordon d'alimentation et clé USB).

⚠ *AVERTISSEMENT*

L'équipement de classe A est destiné à être utilisé dans un environnement industriel. Dans la documentation pour l'utilisateur, une déclaration doit être incluse pour attirer l'attention sur le fait qu'il peut y avoir des difficultés à assurer la compatibilité électromagnétique dans d'autres environnements, en raison de perturbations aussi bien conduisées que radiées.

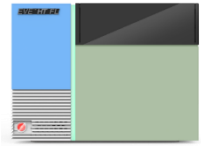
Safety Symbols

The following symbols are found on the medical device and this document. Always use the instrument in the safest possible manner.

Symbol	Meaning
	Caution & Warning
	Protective earth (Ground)
	Power On/Off
	The moving parts symbol indicates areas of the medical device in which moving parts can cause injuries. Do not operate the medical device with the door open.
	This device and consumables conforms to the EC Declaration of Conformity.
	This equipment has been tested and found to comply with the limits for a Class A digital medical device, pursuant to Part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.
	USB Connection
	This product conforms to UL 61010-1, CAN/CSA C22.2 No.61010-1 “Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use, Part I: General Requirements.” This instrument bearing the TÜV symbol are certified by TÜV Product Services to be in conformance with the applicable safety standard for the US and Canada.
	Catalogue number/Reference number
	Serial number
	Manufacturer
	Electrical input
	Consult Instructions for Use An electronic instructions for us (eLFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.

	Disposal of your old appliance 1. When this crossed-out wheeled bin symbol is attached to a product it means the product is covered by the European Directive 2012/19/EU. 2. All electrical and electronic products should be disposed of separately from the municipal waste stream via designated collection facilities appointed by the government or the local authorities. 3. The correct disposal of your old appliance will help prevent potential negative consequences for the environment and human health. 4. For more detailed information about disposal of your old appliance, please contact local distributor, waste disposal service or call the number listed in the manual.
	LED
	Physician. Keep dry Keep away from rain
	Fragile, handle with care
	This way up
	General symbol for recover/recyclable
	Team lift
US Corporation	US Corporation
European Corporation	European Corporation
EC REP	Authorized representative in the European community
UK Representative	Authorized representative in United Kingdom
CH REP	Authorized representative in Switzerland
BRH	Authorized representative in Brazil

Product specifications



EVE™ HT FL	
Analysis time	3 ~ 20 minutes per 48 samples
Measuring range	Detectable range: 1 x 10^4 ~ 2 x 10^7 cells/mL Optimal range: 1 x 10^5 ~ 1 x 10^7 cells/mL
Cell size range	Detectable size : 1 ~ 85 µm (Fluorescence mode) 5 ~ 85 µm (Brightfield mode) Optimal size: 5 ~ 80 µm (Fluorescence mode) 10 ~ 80 µm (Brightfield mode)
Channel	Bright field, Dual fluorescence (AO & DAPI)
Loading sample volume	20 µL per channel
Staining solution	AO/DAPI mixed solution Trypan blue solution Erythrosin B solution
21 CFR Part 11 Option	Available
Operation system	Windows 10
Power	100 ~ 240V, 50/60Hz
Dimensions	586 × 461 × 458 mm (W×D×H)
Weight	61 kg

Ordering information

Cat. No.	Description	Contents
EVE HT FL	High-throughput fluorescence cell counter	Main device 1 ea Desktop & monitor 1 set Multi pipette 1 ea
EVFL-020	EVE HT FL Counting kit	960 tests / kit Counting plate (48 channels × 20 plates) Mixing well plate (96 wells × 10 plates) Reservoir (5 pcs × 4 packs)
EVAD-960	AO/DAPI Staining Solution	20 mL × 2 bottles Acridine orange (AO) & 4',6-diamidino-2-phenylindole(DAPI) stain - Expires 2 months after opening
EVTB-960	Trypan blue Stain	20 mL × 2 bottles Trypan blue Stain solution (0.4%) - Expires 6 months after opening
EVEB-960	Erythrosin B stain	20 mL × 2 bottles Erythrosin B Stain solution (0.05%) - Expires 6 months after opening
EHGQ-001	EVE HT FL QC Plate Fluorescence (optional)	Low level, 1 pc
EHGQ-002	EVE HT FL QC Plate Fluorescence (optional)	Middle level, 1 pc
EHGQ-003	EVE HT FL QC Plate Fluorescence (optional)	High level, 1 pc
EFB-001	EVE HT FL Test Beads (optional)	1 x 1 mL / Pack

Ordering information

Cat. No.	Description	Contents
EHBQ-001	EVE HT FL QC Plate Bright (optional)	Low level, 1 pc
EHBQ-002	EVE HT FL QC Plate Bright (optional)	Middle level, 1 pc
EHBQ-003	EVE HT FL QC Plate Bright (optional)	High level, 1 pc
EHB-001	EVE HT BR Test Beads (optional)	1 x 1 mL / Pack
EHPP-001	Preparation plate (optional)	Preparation plate
EVE HT FL 21 CFR Part 11	EVE HT FL 21 CFR Part 11 software (optional)	21 CFR Part 11 software

Technical support

Visit our Website at **www.nanoentek.com** for:

- Technical resources, including manuals, FAQs, etc.
- Technical support contact information
- Additional product information and special offers

For more information or technical assistance, please call or email.

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EVE™ HT FL

NESMU-EHTFL-001E (V.0.3)

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