CE

ADAMII LS

An image-based fluorescence cell analyzer

User Manual

NanoEntek

ADAMII™ LS User Manual

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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.

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V.0.6 APR 2025

Table of Contents

Introduction

ADAMII™ LS is a versatile fluorescence cell analyzer developed for laboratories in academia and industry. It takes images with one bright field and up to 3 fluorescence channels (GFP, RFP, and DAPI).

ADAMII™ LS allows users to perform several cell-based assays including cell counting, viability measurement, fluorescence expression measurement, apoptosis assay, and cell cycle assay.

ADAMII™ LS is simple to set up and requires little maintenance. With graphical user interface, ADAMII™ LS is quick to learn and easy to use.

[Features]

- Accurate measurement
- Versatile applications
- Offers histograms & dot plots
- Easy to use

Product Components

ADAMII™ LS consists of the following components.

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

ADAMII™ LS

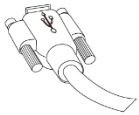
1 EA

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

4 pcs/1 SET

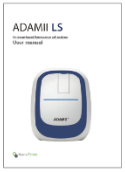
USB connection cable

1 EA



User manual

1 EA



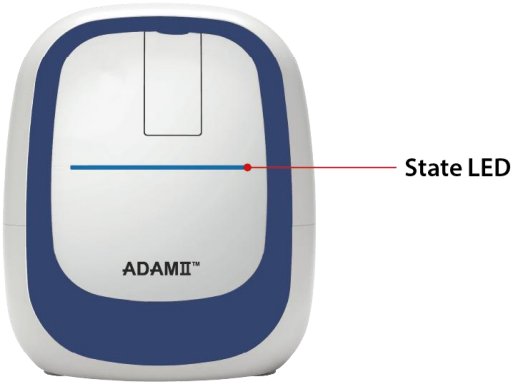
Laptop
(Please note: Laptop will be packaged separately)

1 set

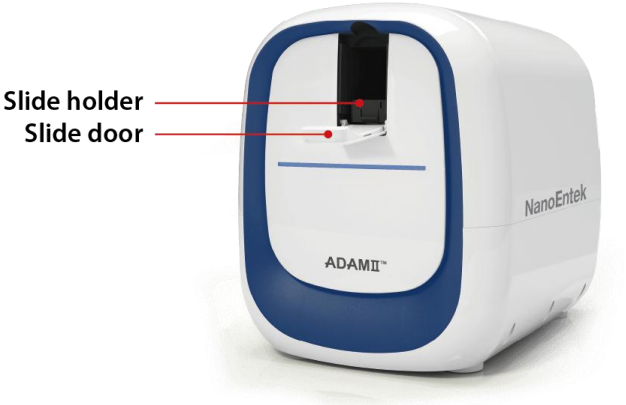
ADAMII™ LS
21 CFR Part Software

Product Description

Front view



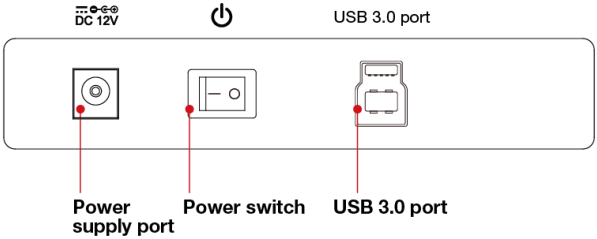
State LED	Blue LED will light up when ADAMII™- LS software is running.



Slide Door	Door to have access to the Slide Holder
Slide holder	Holder to keep ADAMII™ Assay Slide steady

Product Description

Rear view



Power supply port	Connect DC output jack here
Power switch	To turn power On and Off
USB 3.0 port	Connect USB cable here

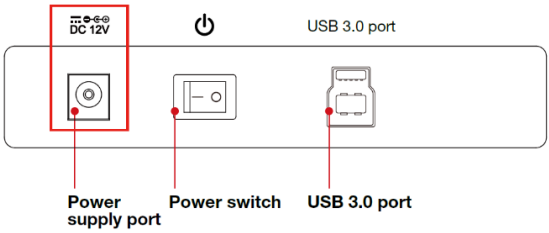
Installation

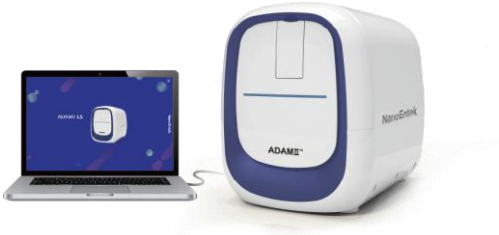
Unpack

1. Open the carton box and remove all foam protectors.
2. Carefully lift the instrument with two hands. Please be advised that the instrument is heavy (19.3kg, 42.5lb).
3. Place the instrument on a flat, stable and level surface.

Connect cables

1. Choose a power cord that fits an electrical outlet. Connect power cord with power adaptor. Plug power cord into an outlet and DC output jack to the power supply port in the back of ADAMII™ LS.
2. Connect USB 3.0 cable to the USB port in the back of ADAMII™ LS and the laptop.



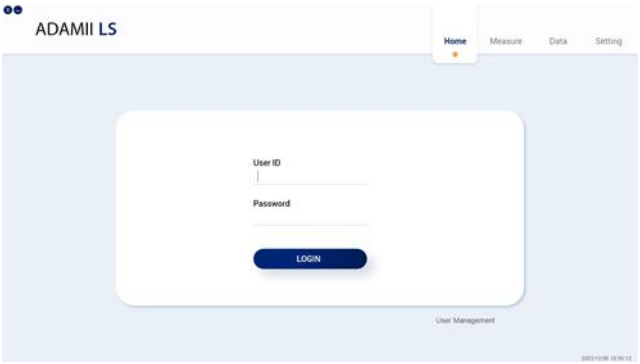


Installation

Run ADAMII™ LS software

1. Turn on the laptop and the ADAMII™ LS instrument.

2. Double click the ADAMII™ LS icon on the Desktop to start the ADAMII™ LS software.



☛ ***Note:*** *When ADAMII™ LS software starts, ADAMII™ LS instrument will go through an initialization cycle. After completion of the initialization, state LED on the instrument will light up as BLUE.*

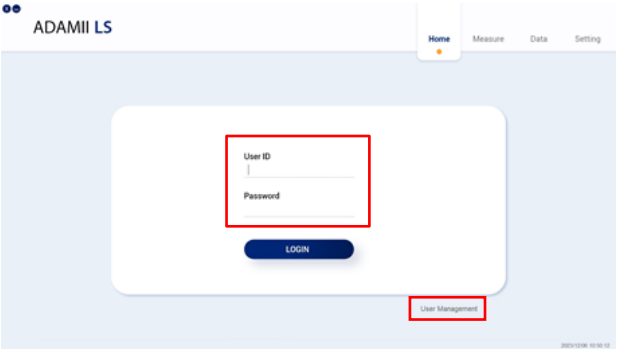
⚠ ***CAUTION:***

Instrument must be turned ON before starting ADAMII™ LS software. If instrument has not been turned on when software starts, software will give a warning.

Home menu

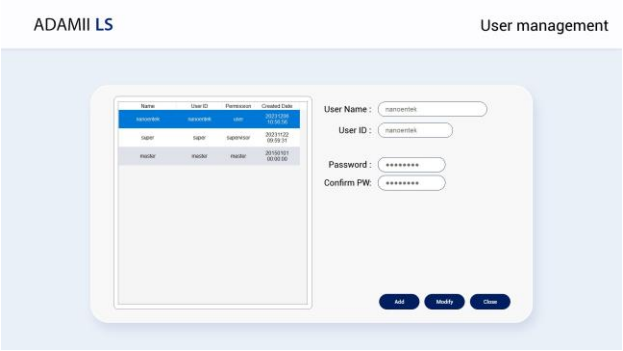
Log in

Default ID and password are both '**master**'.



User management

In '**User management**', one can add new users, change passwords and deleted users. To create a new user ID, click '**Add**' then fill out the blanks (user name, user ID, password, confirm PW).



☛ ***Note**: Only users with admin' level permission can access the '**User management**'.*

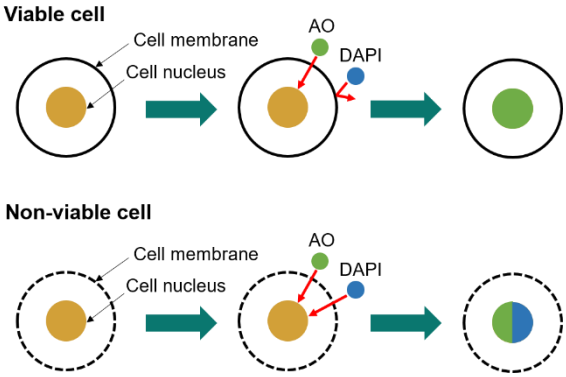
Measurement

Reagents for ADAMII™ LS

ADAMII™ LS can analyze fluorescently labeled samples. In addition, ADAMII™ LS offers 3 types of reagents that come with predefined measurement settings. Details of how to use these reagents are described below.

1) Cell viability reagent

Cell viability reagent is used for Total & Viability analysis. Cell viability reagent is a combination of Acridine Orange (AO), a cell permeable DNA dye, and DAPI, an impermeable DNA dye. Cell counts and viability of the entire sample can be measured by counting cells stained with these reagents. ADAMII™ LS distinguishes viable cells from non-viable cells in the following way.



2) PI cell cycle reagent

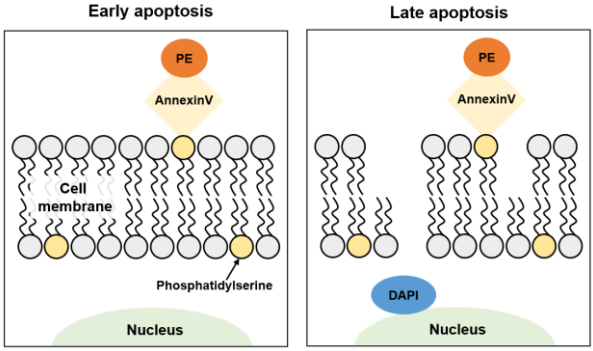
PI (Propidium Iodide) is a reagent often used in cell cycle analysis. PI cell cycle reagent for ADAMII™ LS contains PI and RNaseA. PI cell cycle reagent skips fixation step and stains cells directly. From the images of PI stained cells, ADAMII™ LS can distinguish cells in different phases of cell division cycle (G0/G1, S and G2/M phases) by precisely analyzing the intensities of PI stained cells.

Measurement

Reagents for ADAMII™ LS

3) Apoptosis detection kit

To analyze apoptosis, ADAMII™ LS uses AnnexinV conjugated with phycoerythrin (PE) and DAPI. AnnexinV is commonly used to detect apoptotic cells. In early phase of apoptosis, phosphatidylserine (PS) which is a component of cell membrane and normally resides on the cytosolic side of cell membrane, becomes exposed to the outer side of cell membrane. AnnexinV specifically binds to PS exposed on the outer side of cells and PE attached to AnnexinV makes apoptotic cells fluorescence. As apoptosis progresses and the integrity of cell membrane collapses, cell membrane becomes porous and DAPI can diffuse into cells and stains cell nucleus. Based on the intensities of PE and DAPI, ADAMII™ LS quantifies percentages of cells in early and late phases of apoptosis.



Measurement

Sample preparation

As mentioned in the previous chapter, ADAMII™ LS has 3 types of standardized reagents.

- **Cell viability reagent** for Total & Viability assay
- **PI cell cycle reagent** for Cell cycle assay
- **Apoptosis detection kit** for Apoptosis assay

Brief steps to use ADAMII™ LS reagent kits are explained below.

☛ ***Note****: If you use your own samples (such as GFP expressing cells, cells stained with fluorescence dyes, etc), skip this part and go directly to* ***'Load sample'*** *step on the next page.*

A. Cell viability reagent (Cat.no ALAD-100)

1. Recommended cell concentration for Total & Viability assay is 5 x 10^4 ~ 5 x 10^6 cells/mL.
2. Make a 100 µL aliquot of sample.
3. Add 10 µL of cell viability reagent (Gently vortex the reagent tube before using it).
4. Mix well (Gently vortex or tap with finger a few times).

B. PI cell cycle reagent (Cat.no ALPI-100)

1. Recommended cell concentration for Cell cycle assay is around 3 x 10^6 cells/mL.
2. Prepare a sample in PBS (phosphate buffered saline).
3. Make a 25 µL aliquot of the sample.
4. Add 25 µL of PI cell cycle reagent to the aliquot.
5. Mix well by pipetting up and down a few times (Do not vortex! Vortex may cause cells to clump together and affect the assay).

☛ ***Note****: For accurate and reliable results in cell cycle assay, it is recommended to analyze 75 frames using a 1 channel assay slide.*

Measurement

Sample preparation

C. Apoptosis detection kit (Cat.no ALAP-100)

1. Recommended number of cells for Apoptosis assay is1 x 10^6 cells per 1 test.
2. Prepare cell samples with or without prior apoptosis inducing treatments.
3. Wash out cell culture media in the aliquots with PBS once.
4. Centrifuge the aliquots and remove PBS.
5. Resuspend cells with 100 µL of 1X AnnexinV binding buffer in Apoptosis detection kit and mix well (Gently vortex or tap with finger a few times).

☛***Note****: The 10X binding buffer included in the kit must be diluted with DW to a final concentration of 1X before use.*

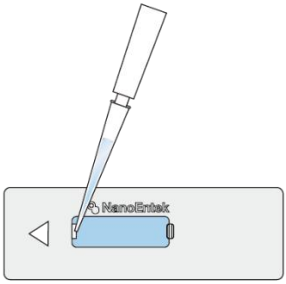
6. Add 5 µL AnnexinV-PE reagent included in Apoptosis detection kit and mix well (Gently vortex or tap with finger a few times).
7. Incubate cells at room temperature for 15 min. (Avoid light after adding AnnexinV-PE)
8. Centrifuge and remove supernatant.
9. Resuspend cells with 500 µL of AnnexinV binding buffer and add 1.25 µL DAPI solution. (Keep test-tubes at 4℃ after adding DAPI solution)

Measurement

Load sample

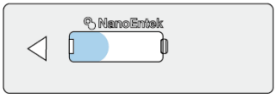
To measure sample with ADAMII™ LS, you must use **ADAMII™ Assay Slide**.

1. Load **25 µL** of prepared sample into an ADAMII™ Assay Slide.

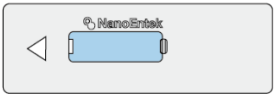


☛ ***Note****: Before loading the prepared sample into ADAMII Assay Slide, please mix the sample thoroughly.*

☛ ***Note****: Load 25 µL to fill the channel in ADAMII Assay Slide. Load sample at a slow and constant speed to avoid bubbles as bubbles may have interfere with image analysis.*



<Low volume>



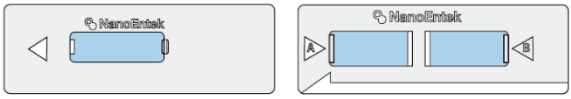
<Correct volume>

2. After loading sample, place ADAMII Assay Slide on a flat surface for **1 minute** to let the sample settle down.

Measurement

Load sample

In addition to 1 channel slides, 2 channel slides that can measure two samples at once are also available. After loading sample into two chambers, user can select assay for each channel. Please refer to the chart below for the differences between one and two channel slides.

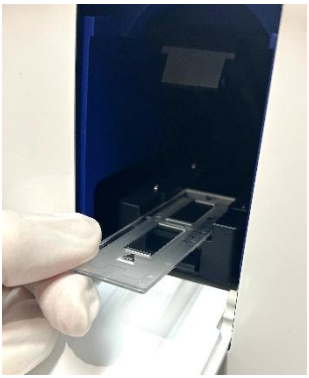


<1 channel slide> <2 channel slide>

	1ch slide	2ch slide
Loading volume	25 µL	25 µL (for each chamber)
Number of frame	15 ~ 75	11 ~ 55 (for each chamber)
Measuring time **(When selecting the maximum number of frames, Total & Viability assay)**	about 4 min 20 sec	about 6 min 45 sec

Measurement

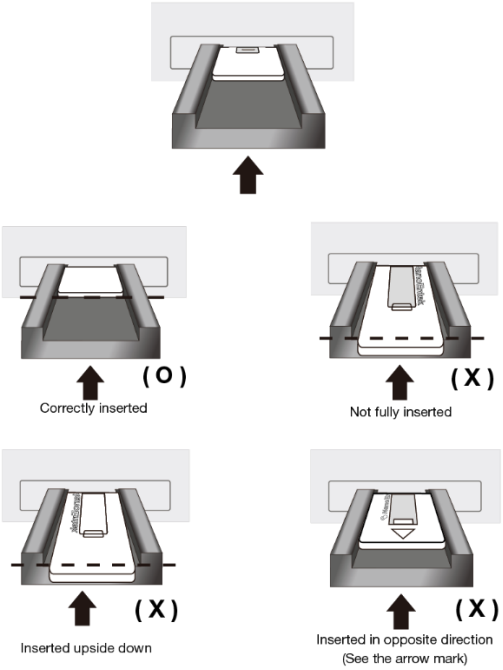
Insert assay slide



1. Find small gap on the top of the “white” door in front of ADAMII™ LS and open the door.
2. Find “black” slide holder and a push bar in front of the slide holder. Push the bar to open the slide holder.
3. Insert ADAMII Assay Slide and push the slide all the way in. --
4. Press down the cap of the slide holder until you hear “click” sound.
5. Close the “white” door.

⚠ ***CAUTION:***

Illustrations of various cases of erroneous Assay Slide insertion are presented below.

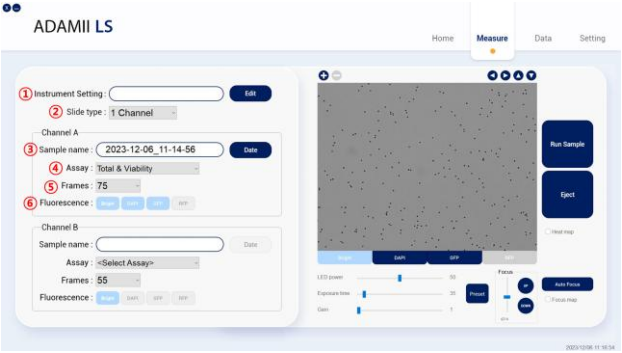


Measurement

Measure setting

Go to “Measure” tab by clicking “Measure” on the upper right corner of ADAMII™ LS software.

In the “Measure” tab, on the left side, there are several slots to enter sample information and options to choose from.



① **Instrument setting**: If you have previously saved settings, you can load a saved setting. Detailed steps to save and modify Instrument setting can be found on the next page.

② **Slide type**: Choose 1 Channel for single channel ADAMII Assay Slide or 2 Channel for dual channel ADAMII Assay Slide.

③ **Sample name**: Enter your sample name. If you click the “Date” button, current date and time will be filled automatically and used as a sample name.

④ **Assay**: Choose assay type and number of frames. There are currently 5 assays to choose from (Quick count, Total & Viability, Cell cycle, Apoptosis, and Fluorescence expression).

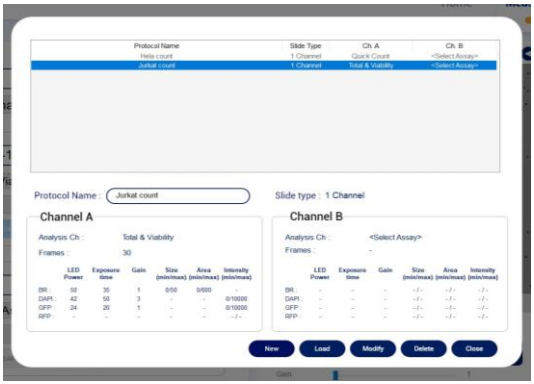
⑤ **Frames**: You can choose from minimum 15 to 75 frames in 15 frame unit (1ch slide) or 11 to 55 frames in 11 frame unit (2ch slide).

⑥ **Fluorescence**: Choose up to 2 Fluorescence channels (for Fluorescence expression assay only).

Measurement

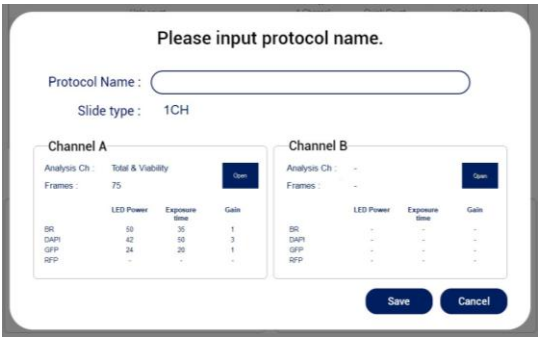
Measure setting

▶Instrument setting



Instrument setting can be saved and recalled for future measurements.

1. Click **'Edit'** button to load or modify a saved protocol. (Refer to the ① on the image presented at page 19)
2. Select a protocol from the list of saved protocols and click **'Load'** to load the camera settings.
3. Change a protocol name and click **'Modify'** to rename it.
4. To delete a protocol, select a protocol and click **'Delete'** button.
5. Click '**New**' button to create new setting protocol.



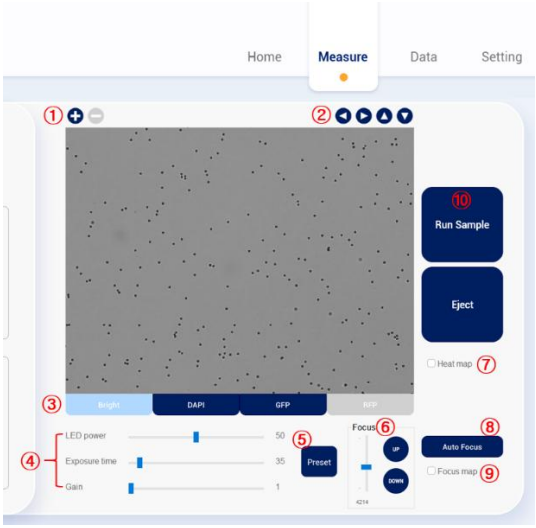
6. Click '**Open**' to import camera settings from saved data.
7. Enter a protocol name and click '**Save**'.

Measurement

Measure setting

▶ Camera setting

After inserting ADAMII Assay Slide, click the **'Insert sample'** button to bring the slide holder to the imaging position. After the slide holder is at the imaging position, live image from camera will be shown in the window on the right side.



① Click **'+' or '-'** button to zoom in or out.

② Click one of the **'arrow'** buttons to move in x and y directions.

③ Click **'channel'** button to switch between 4 channels (Bright field, DAPI, GFP and RFP channels). A gray button means that particular channel is disabled.

④ **LED power, Exposure time, Gain**: Change the strength of light source, exposure time of camera, or gain of camera to adjust brightness and contrast of image.

⑤ Click **'Preset'** button to restore LED power, Exposure time and Gain to the default values

⑥ Click **'Up'** or **'Down'** button to change **Focus.** Please refer to the next page for recommended focus setting.

⑦ Click **'Heat map'** to detect overexpression of cells.

⑧ Click **'Auto Focus'** to find suitable focus automatically.

Measure setting

▶ Camera setting

⑨ Click **'Focus map'** to generate a focus map created by calculating the focus difference between the first and last frames. When you check the focus map and run auto focus, the focus is measured at both ends of the frame and a focus map is created based on the results to be used in actual running.

⑩ Click **'Run Sample'** button to start capturing images.

☞ ***Note****: Slide bars can also be adjusted with the arrow buttons on the keyboard or by scrolling the mouse over the slide bar.*

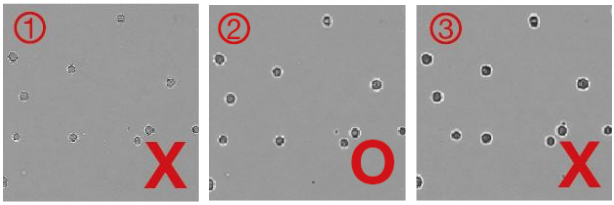
Measurement

Measure setting

▶ **Focus/ intensity example**

1. Focusing Guide

[Bright]

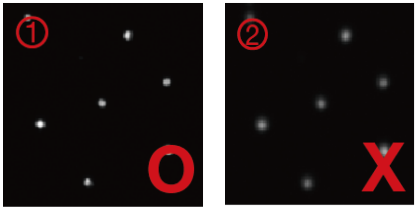


① The outlines of cells are clear BUT the intensity of cells is not easily distinguishable from the intensity of the background.

② The outlines of cells are clear AND the intensity of cells is easily distinguishable from the intensity of the background.

③ While the intensity of cells is easily distinguishable from the intensity of the background, the outlines of cells are too thick and cell sizes could be measured bigger than real values.

[Fluorescence]



① Boundaries of cells are clear and crisp.

② Boundaries of cells are blurry.

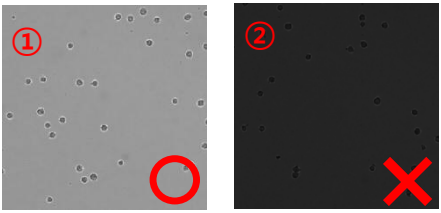
Measurement

Measure setting

▶ Focus/ intensity example

2. Intensity

[Bright]



① Cells can easily be identified.

② Image is too dark and cells are not easily identified.

[Fluorescence]

① Most cells are visible and fluorescent signals are not saturated. Taking images under complete saturation is important for Cell cycle assay and Fluorescence expression assay.

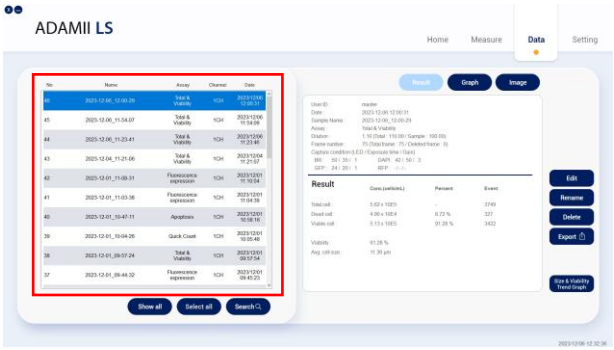
② Most cells are visible but fluorescent signals are saturated. While this kind of images can be used for Total & Viability assay, they are not suitable for Cell cycle assay or Fluorescence expression assay.

③ Image is too bright and all of fluorescent signals are fully saturated.

Data

Data display

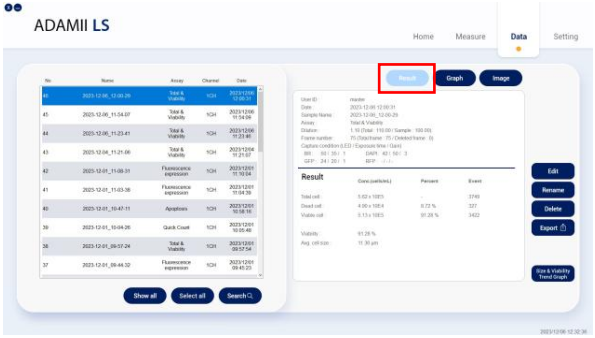
After running an assay, ADAMII™ LS automatically adds results in the **Data** tab. Choose a data entry to review the results. Click one of “Result”, “Graph”, or “Image” button on the top right to check summary of the results, graphs, and raw images. To edit gating conditions for the result, click ‘Edit’ button on the right side.



Data is displayed in a chronological order. One can search data using a user name or a type of assays. Multiple data entries can be selected by choosing entries while pressing the ctrl or shift keys on the keyboard.

1. Result

In the result tab, the sample information will be shown at the top and results of cell counts or analysis at the bottom.

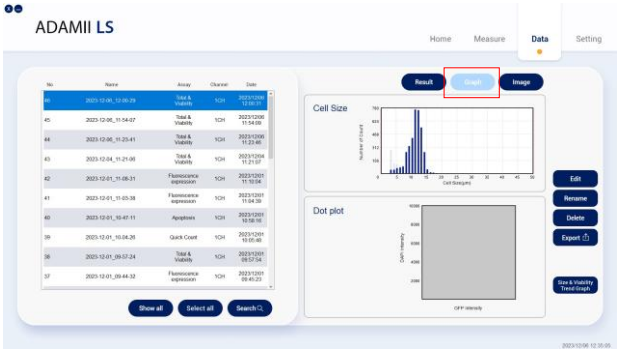


Data

Data display

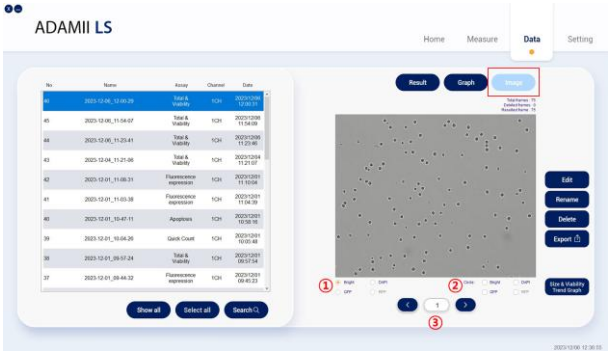
2. Graph

In the graph tab, a cell size histogram and/or a dot plot will be displayed.



3. Image

In the image tab, raw images will be displayed.



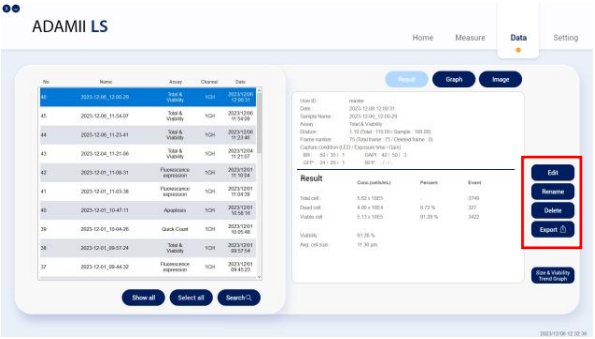
① Click a channel to see images from that channel.

② Click a channel for “Circle” to display circular markers over image to identify those cells that have been detected in each channel. Multiple channels for “Circle” can be selected.

③ Click the left or right arrow to see images taken at a different position. Enter a number to go to a specific frame.

Data

Data edit



① **Edit**: In edit menu, one can change gating for size or intensity histograms, or for a dot plot. For details, refer to the '**Data analysis**' section on the next page.

② **Rename**: To rename a data entry, select a data entry and click '**Rename**' button to change the name of the selected data.

③ **Delete**: To delete a data entry, select a data entry and click '**Delete**' button.

☛ ***Note****: Once deleted, the data cannot be recovered.*

④ **Export**: ADAMII™ LS allows you to export data in 4 different formats; JPG, Excel, PDF, and FCS. After setting a path and data formats, click '**Save**' button.

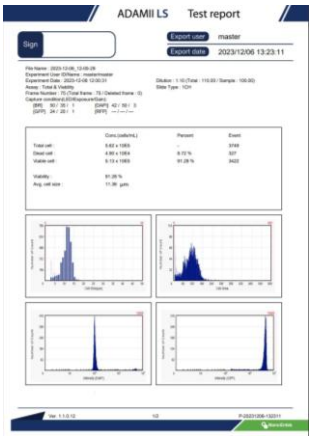
☛ ***Note****: In Quick Count assay and Total & Viability assay, data is not allowed to export in FCS format.*

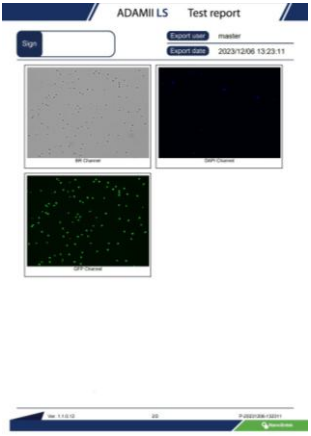
- **JPG**: Raw images of bright and fluorescence channels.
- **Excel** (csv, xls): Data table containing comprehensive information in a spreadsheet.
- **PDF**: A report summarizing data such as analyzed results and graphs. Refer to the following page for examples of the reports.
- **FCS**: Data in flow cytometry standard format for analyzing data using a third-party program.

Data

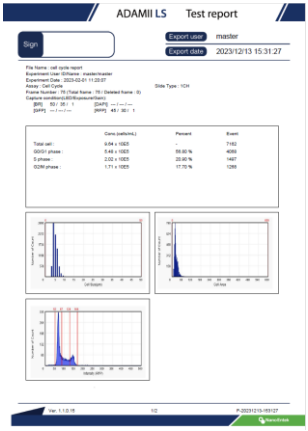
Data edit

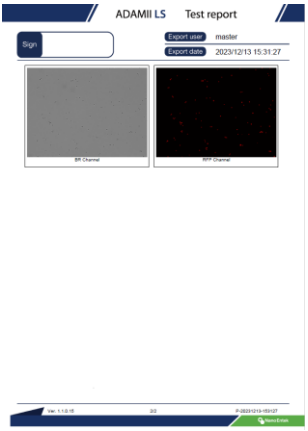
[Total & Viability]





[Cell cycle]





Data analysis

▶ Common feasures of analysis

Analysis is an important step in ADAMII™ LS to acquire accurate and consistent results. Depending on how to set analysis parameters, results may vary. Therefore, it is recommended that you analyze several experimental data before choosing final sets of analysis parameters.

Currently, ADAMII™ LS offers 5 assays. Common features of analysis for all 5 assays will be explained first, and assay specific features will be described next.

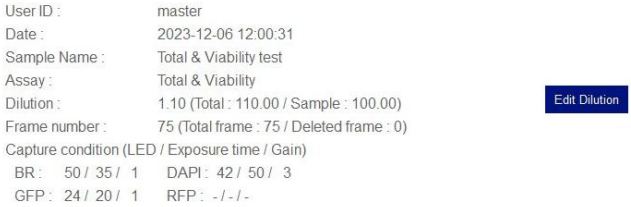
If you pick a data entry and press the **Edit** button (on page 27), a screen like the picture above will appear. The right part shows the same information as shown in “Result”. On the left side, there are several gating options you can change including “Size”, “Area” or “Intensity” of cells. When these gatings are changed, “Result” on the right will be automatically updated.

Data

Data analysis

▶ Common features of analysis

1. Information



- **User ID**: the user ID of whom ran this measurement
- **Data**: the date and time when this measurement was start
- **Sample Name**: the sample name that was entered during measurement
- **Assay**: the type of assay that was chosen for this measurement
- **Dilution**: the dilution factor of the sample. If samples have been diluted to adjust cell concentrations to match recommended concentration, please “Edit Dilution” and enter dilution factor. *(All the dilutions happening during standard sample preparation should NOT be taken into consideration. They will be applied automatically).*
- **Frame number**: the number of images that ADAMII™ LS used for final results
  - Total frame: the number of images that ADAMII™ LS took.
  - Deleted frame: the number of images that were excluded from data analysis. *(How to “Delete” frame can be found in the later part of this “Data analysis” section).*
- **Capture condition**: all the camera settings that were used for image acquisition. LED power (in %), exposure time (in msec), and gain for each channel.

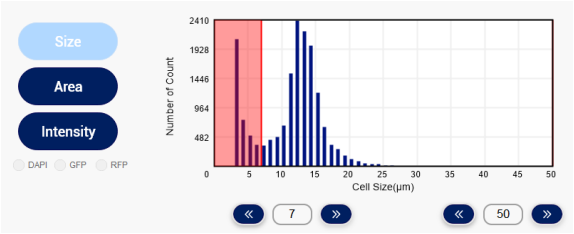
Data

Data analysis

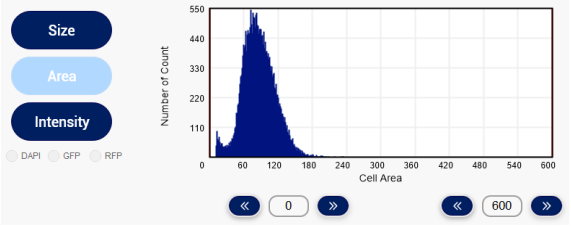
▶ Common features of analysis

2. Graph

ADAMII™ LS offers 4 types of graphs.



- **Size histogram**: a histogram of cell size measured from bright field images. The minimum and maximum size of cells can be set either by entering numbers (0 ~ 50 μm) or moving boundaries by clicking left or right double-arrows. Excluded cell sizes will be displayed in red. Using this function, small or large objects can be gated out.

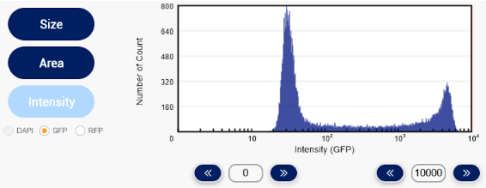


- **Area histogram**: a histogram of cell area measured from bright field images. The minimum and maximum size of cells can be set either by entering numbers (0 ~ 600 pixel) or moving boundaries by clicking left or right double-arrows. Excluded cell areas will be displayed in red. Using this function, small or large objects can be gated out.

Data

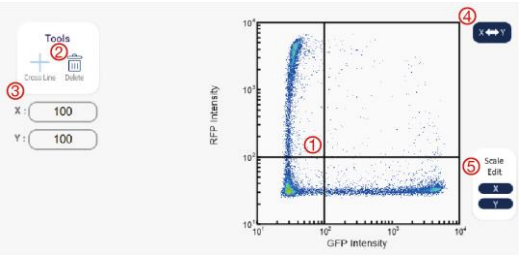
Data analysis

▶ Common features of analysis



- **Intensity histogram**: histograms of cell intensities measured from fluorescence channels. The x-axis is in a log scale. Similar to cell size or area histograms, one can exclude cells with low or high intensities.

  ☞ *Note: In the case of single channel fluorescence expression assay, the gating of the intensity graph is used as a tool to analyze the expression level. See page 40 for details.*



- **Dot plot**: a dot plot of cell intensities. This is only available for Apoptosis assay and 2 color Fluorescence expression assay.

  ① Click on any part of the dot plot or click the '+' button to create a cross line. Grab either horizontal or vertical line to move the lines.

  ② Click 'Delete' button to remove the cross line.

  ③ Enter values to adjust the positions of the cross line more precisely

  ④ Click 'X ↔ Y' button to swap X-axis with Y-axis.

  ⑤ Click 'Scale Edit' to change the scale of each axis. Clicking the button will change the scale in the order of 1, 10^1, 10^2, and 10^3.

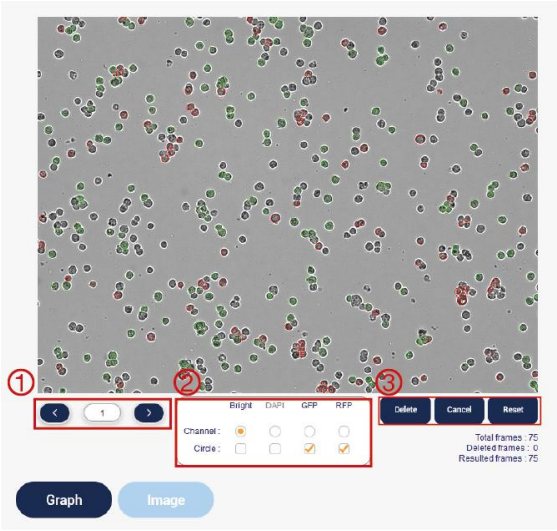
Data

Data analysis

▶ Common features of analysis

3. Image

Click ‘Image’ button on the lower left corner to see raw images.



① Enter a frame number or click the arrows to see raw images.

② Choose a ‘Channel’ to see raw image of the channel. Choose ‘Circle’ of one or more channels to toggle on the circular markers that were counted in each channel.

③ In a frame, if there are debris or cells from different channels that do not match each other (examples shown below), one may exclude the frame by clicking ‘Delete’

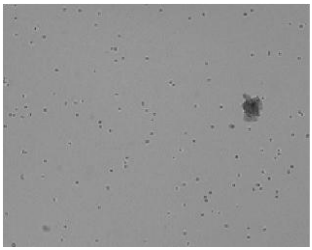
- **Delete**: to exclude a frame from data analysis
- **Cancel**: to restore a deleted frame to be included for analysis
- **Reset**: to restore all deleted frames

Data

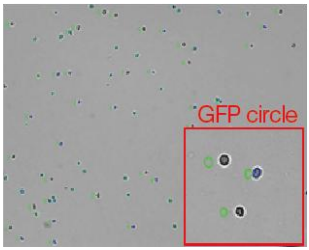
Data analysis

▶ Common features of analysis

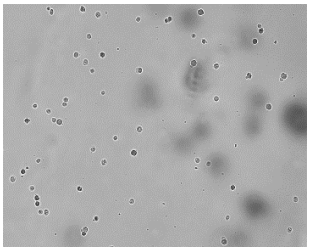
☛ ***Note***: *Examples of images to be deleted.*



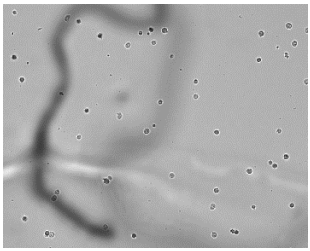
[Debris]



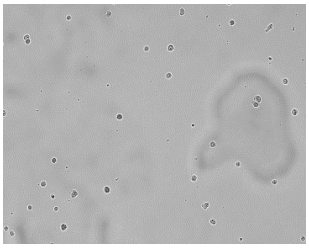
[Mismatched]



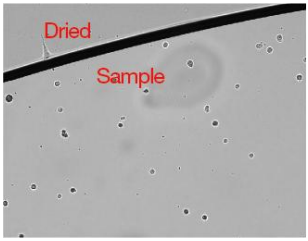
[Dust]



[Dust]



[Liquid stain]



[Dried sample]

Data

Data analysis

▶ Quick count

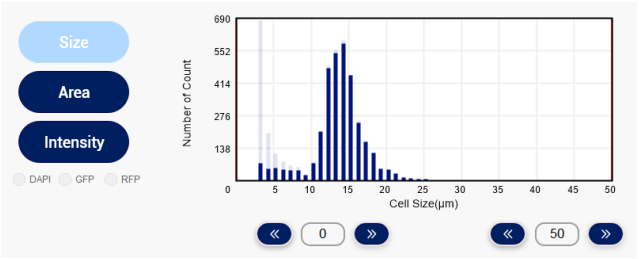
Quick count is an assay that measures total cell counts only using bright field images.

☛ ***Note****: While ADAMII™ LS software is designed to exclude most of dust from counting, there is a possibility that dust can be counted as cells if only bright field images are used for cell counting.*

Data analysis

▶ Total & Viability

Total & Viability is an assay that counts total cell counts and dead cell counts using Acridine Orange (AO) and DAPI. AO is membrane permeable and stains every cells. DAPI is membrane impermeable and only stains dead cells. For accurate counting, only those cells that are identified both in bright field image and AO channel will be counted for total cell counts. Cells identified in all three channels, bright field, AO and DAPI channel, will be counted as dead cells.

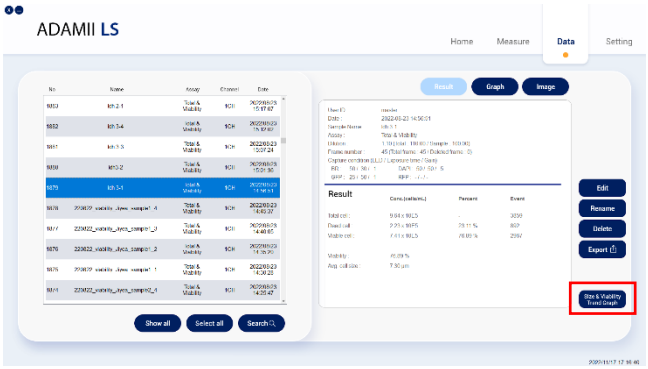


Due to this feature, the size and area histograms of Total & Viability assay will be presented in two different colors. Light blue histogram is for all the objects found in bright field images including debris. Dark blue histogram is for cells that have been found in both bright field and AO images.

Data

Data analysis

▶ Total & Viability



If there are multiple Total & Viability data from a same sample, ADAMII™ LS can generate graphs to show trends in cell size and viability. Click **Size & Viability Trend Graph** in the **Data menu**.

On the left, the lists will show all the data entries measured by Total & Viability assay in a chronological order. Select data to evaluate trends to generate trend graphs of cell size and viability. Click **'Export'** button to save trends as a PDF report.

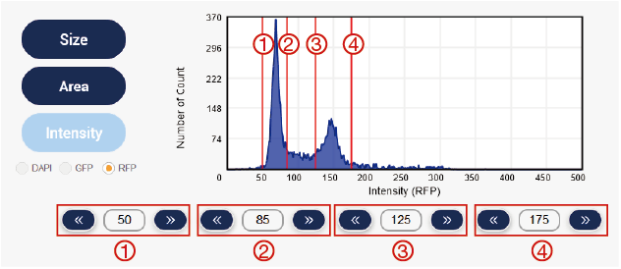
Data

Data analysis

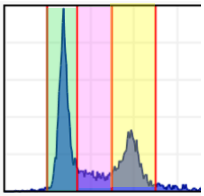
▶ Cell cycle

Cell cycle assay is an assay that quantifies the percentages of cells in each of phases in cell cycle. The analysis is based on the principle that DNA contents in cells change depending on the phases of cell cycle. Cells in S phase, where DNA is synthesized, have more DNA than cells in G0/G1 phase. In G2/M phase, when cells have completed DNA synthesis, the amount of DNA in cells is roughly double the amount in cells in G0/G1 phase. To quantify DNA contents, **Cell cycle assay** uses Propidium Iodide (PI) which is a commonly used fluorescence dye known to intercalate with DNA regardless of nucleotide sequences. Since PI intensity is proportional to DNA content, PI intensity in a cell can be used as an indirect measure of the amount of DNA content.

When measuring mammalian cells, PI intensity histogram typically shows two peaks as shown below.



Intensity graph of Cell cycle assay has 4 gating bars (①~④). Adjust the gating bars to position each peak in the middle of ①~② or ③~④. The gating bar can be moved by entering the numbers or clicking the arrows. Cells within ①~② are in G0/G1 phase and those within ③~④ are in G2/M phase. Cells within ②~③ are in S phase. Please refer to the following.



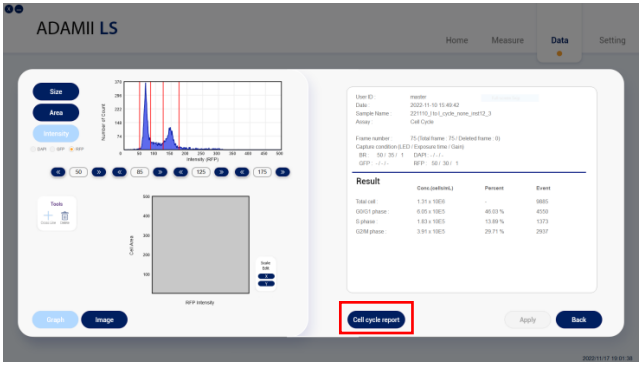
Result

	Conc.(cells/mL)	Percent
Total cell :	9.13 x 10E5	-
G0/G1 phase :	4.50 x 10E5	49.35 %
S phase :	2.34 x 10E5	25.59 %
G2/M phase :	1.82 x 10E5	19.91 %

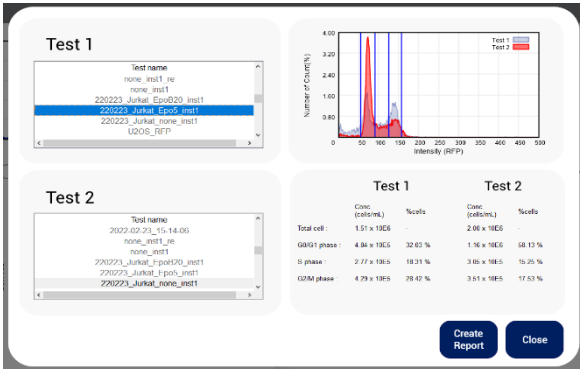
Data

Data analysis

▶ Cell cycle



In general, Cell cycle assay compares two or more groups of samples in which one is usually control sample. The intensity histograms of PI from two samples can be analyzed using the same gating conditions so that the percentage values of two groups can be compared with each other to quantify effects of perturbations or the lack of them. ADAMII™ LS offers an option to create a PDF report with comparison results. One can access this option by clicking the 'Cell cycle report' button in the Edit window.



1. Select two data sets to compare from the list on the left.
2. Adjust the 4 blue gating bars and results will be updated real time.
3. When gating positions are determined, click the "Create Report" button.
4. In 'save path', enter file location to save cell cycle report in PDF format.

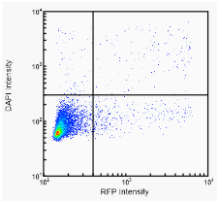
Data

Data analysis

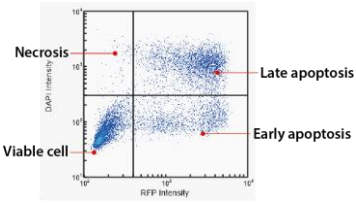
▶ **Apoptosis**

AnnexinV is the most frequently used protein to detect apoptotic cells. AnnexinV is often used along with nuclear dyes (DAPI, PI, 7-AAD, etc.) to distinguish early and late apoptosis. ADAMII™ LS's **Apoptosis** assay uses a combination of AnnexinV labeled PE (Phycoerythrin) and DAPI (4′,6-diamidino-2-phenylindole). For detailed staining methods, refer to the sample preparation in page 15.

Typical data would look like dot plots shown below.



[Negative control]



[Apoptosis induced]

AnnexinV-PE and DAPI dot plot can be divided into four sections; viable cell, early apoptosis, late apoptosis and necrosis. In an early stage of apoptosis, only AnnexinV is positive and these cells are on the lower right quadrant. When apoptosis progresses, DAPI also becomes positive and these cells are on the upper right quadrant. User can move both horizontal and vertical lines by grabbing a line with mouse or entering intensity values.

☛ ***Note****: If it is difficult to set the position of these cross lines, first create a cross line in the negative control and use the same positions for apoptosis induced samples.*

Data

Data analysis

▶ Fluorescence expression

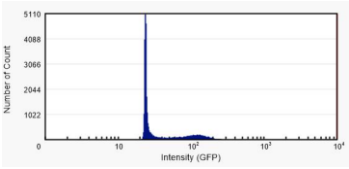
Fluorescence expression assay is maybe the most versatile function of ADAMII™ LS. There is no reagent or a predefined protocol. Users can use this Fluorescence expression assay to quantify fluorescence intensity distributions in many contexts including but not limited to measuring drug treatments, phagocytosis, GFP expressions, RFP expressions, protein updates, etc. ADAMII™ LS is capable of measuring intensity histogram in 3 colors: DAPI, GFP, and RFP. In addition, two of these fluorescent colors can be measured and analyzed simultaneously.

☛ ***Note****: fluorescence wavelength in ADAMII™ LS.*

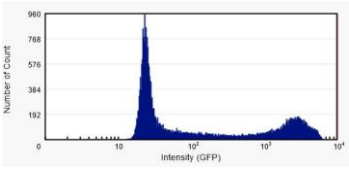
	Excitation	**Emission**
DAPI	378nm	447nm
GFP	466nm	525nm
RFP	543nm	561nm

1. Single channel

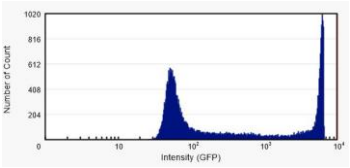
When one measures fluorescence intensity, adjusting camera conditions to the optimum setting is very important. Fluorescence signals cannot be too week but also cannot be too saturated.



▶ Too weak to distinguish



▶ Good example



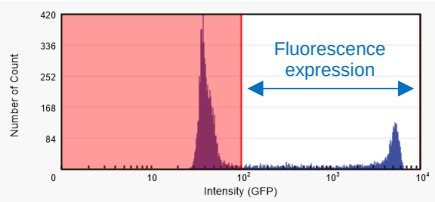
▶ Saturated because it was too strong

Data

Data analysis

▶ Fluorescence expression

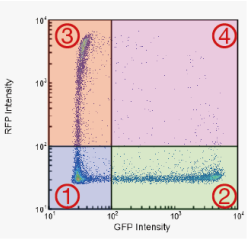
When measuring fluorescence intensity in one channel, user has to choose one gating value and this gating condition will be used to quantify the percentage of fluorescence expression.



The intensities lower than the gating condition will be shown in red. And the remaining part will be considered to be positive in fluorescence expression.

2. Double channels

ADAMII™ LS can measure up to two fluorescence intensities simultaneously in one measurement. After taking images, intensities will be shown in a dot plot. Users can move the cross lines to divide data into 4 quadrants. The following example is a dot plot after taking GFP and RFP channels.



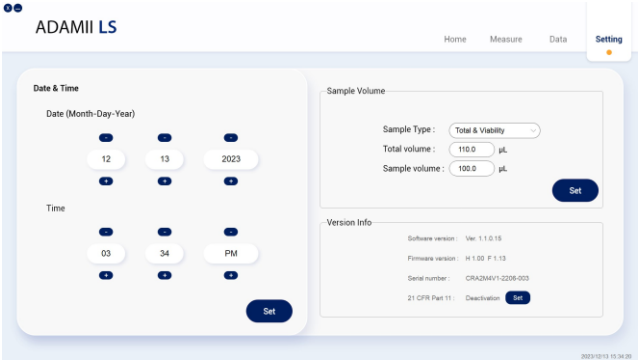
Result

	Conc.(cells/mL)	Percent
Total cell :	1.72 x 10E6	-
GFP expression :	5.06 x 10E5	29.45 %
RFP expression :	5.72 x 10E5	33.34 %
Dual positive :	2.11 x 10E4	1.23 %
None expression :	6.18 x 10E5	35.98 %
GFP Transfection	30.68 %	
RFP Transfection	34.57 %	
Avg. cell size :	13.41 μm	

GFP expression (②) means cells expressing only GFP. Similarly, **RFP expression** (③) means cells expressing only RFP. And **Dual positive** (④) refers to cells expressing both GFP and RFP. Note that these quadrants are marked by different colors. **GFP Transfection** refers to all cells expressing GFP. Therefore, it is expressed as a value of ②+④. Likewise, **RFP Transfection** is expressed in all cells expressing RFP, thus representing a value of ③+④.

Setting

Setting menu



Date & Time: Users can change the date and time by pressing “-” and “+” buttons.

Sample Volume: Users can change the dilution factor by entering the initial sample volume (Sample volume) and the final volume after completion of sample preparation (Total volume).

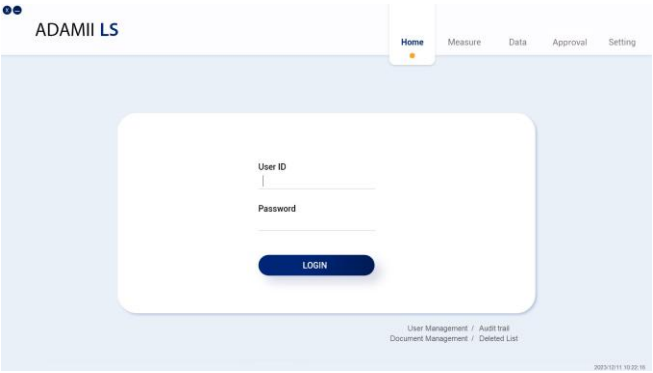
Version info: Displays the versions of the software and firmware.

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 the part 11 of the Title 21 of the Code of Federal Regulations, specifically 21 CFR Part 11. ADAMII LS offers 21 CFR part 11 compliance option which is particularly important for cGMP labs or facilities. Please contact technical support to add this option to ADAMII LS.

21 CFR part 11 activation

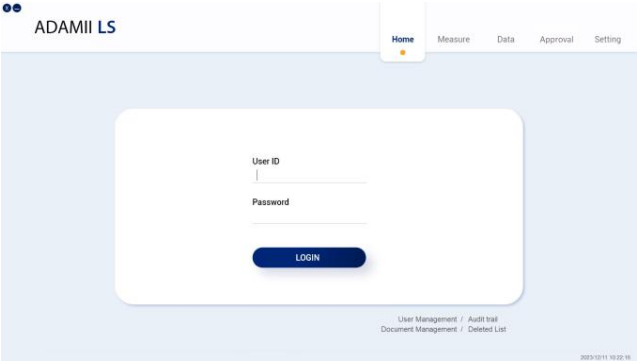
Once “21 CFR part 11” has been activated, in the lower right corner in “Home” tab, in addition to the standard menu of “User Management”, three menus have been added – “Audit trail, Document list, and Deleted list”.



21 CFR part 11

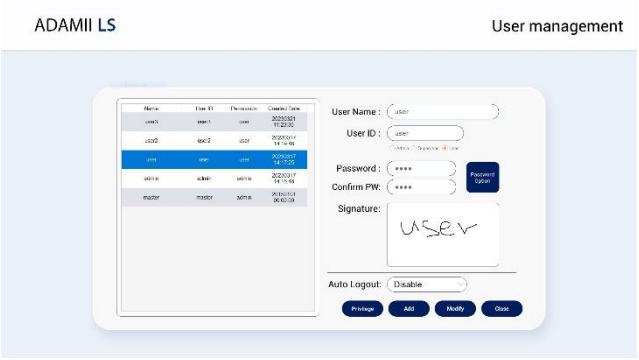
Log In

Log in from login screen. In the lower right corner, there are 4 menus – User Management, Audit trail, Document list, and Deleted list.



User management

When the 21 CFR part 11 program is activated, several new functions are added to the User management menu. While all users have permission to access this menu, only those users with permission can make any changes.

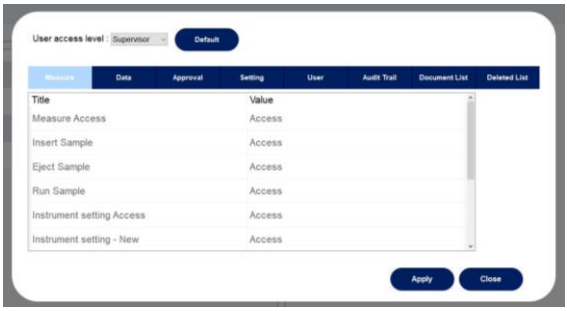


21 CFR part 11

User management

1) Privilege

There are three types of users (Admin, Supervisor, User) available with different levels of privileges. For the default lists of allowed privileges for each type of users, refer to the “ADAMII LS 21 CFR part 11 Support” document’s appendix. Admin is allowed to change permissions for each user including Supervisor.



2) Password option

Set the password management rules.

① Change cycles (Disable, 30 days, 90 days, 180 days): the duration before mandatory change of passwords.

② Account lock (Disable, 3 times, 5 times, 10 times, 15 times): the number of allowed attempts before locking account

③ Minimum length (Disable, 3 or more, 5 or more, 10 or more, 15 or more): minimum length of passwords

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User management

④ Special characters (Disable, Enable): option to impose mandatory usage of special character

⑤ Uppercase (Disable, Enable): option to impose mandatory usage of uppercase character

⑥ Reuse (Disable, 30 days, 90 days, 180 days): the duration of using a same password

3) Lock in user list

User ID is locked when login attempts fail more than the number defined in the “Account Lock”. Locked user is displayed in red. Place mouse curser on a user ID and right-click to lock or unlock the user ID. Only authorized users can use Lock/Unlock function.

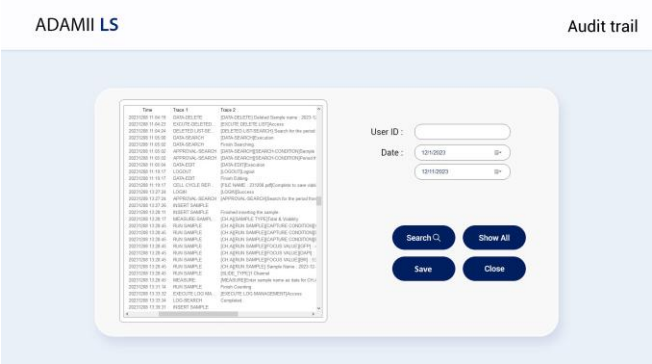
21 CFR part 11

Audit trail, Document manage, Delete list

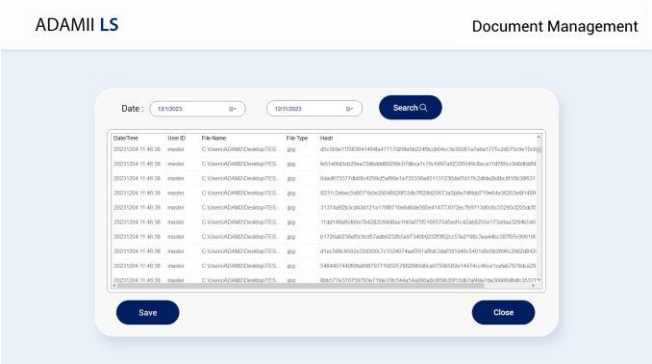
1. Audit trail

This Audit trail shows the lists of allows you to view a list of instrument logs.

- Every Logs can be saved in CSV file format.



2. Document manage

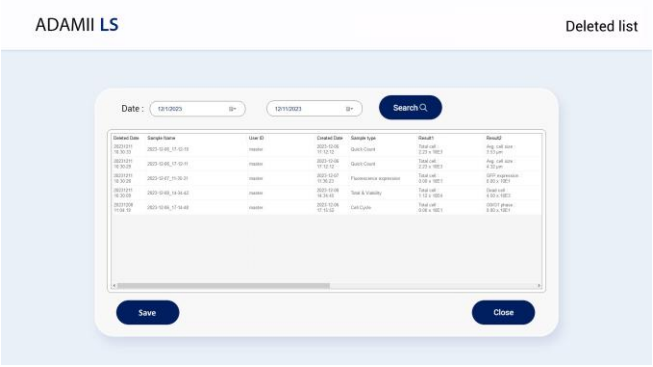


This Document manage shows the lists of exported documents, allows you to check the documents made in the instrument.

21 CFR part 11

Audit trail, Document manage, Delete list

3. Deleted list

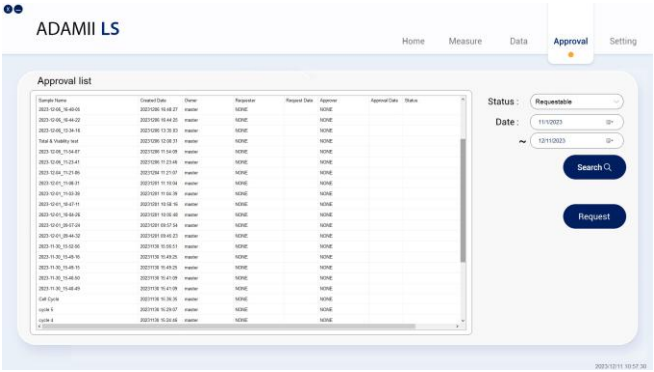


Deleted list shows the lists of data that have been deleted.

21 CFR part 11

Approval

In the approval tab, users can request an approval for a data.



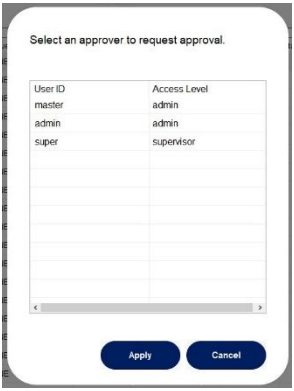
Depending on the approval state, data lists will show one of four statuses (Requestable, Requesting, Approvable, Approved).

1. **Requestable**

The list shows data that can be requested for an approval.

① Select the data to get an approval.

② Click the **'Request'** button.



③ Select an approver to send the request to.

④ Click the **'Apply'** button.

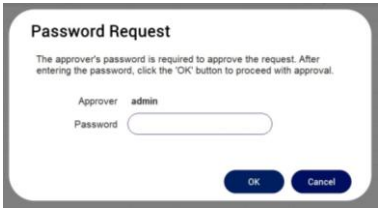
Approval

2. **Requesting**

The “Requesting” lists will show the data that have been requested but not been approved yet. It is possible to **‘Cancel’** the request. Approver can approve the request even without logging-in to the system.

① Select the Data in list

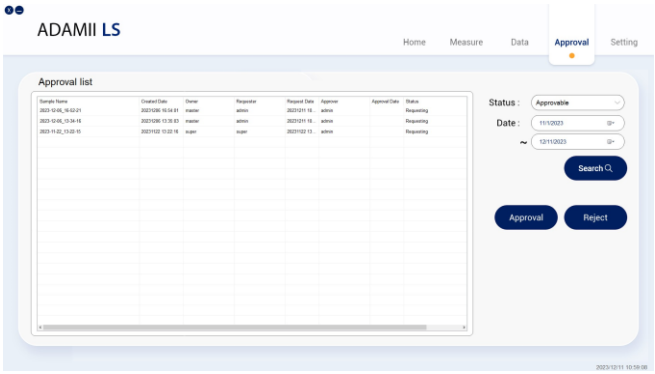
② Click the **‘Approval’**



③ Enter the password to approve data.

3. **Approvable**

If a user has received request(s) for approval, the requested data will be shown in the “The approvable list”. A user can approve or reject the data by choosing **‘Approval’** or **‘Reject’**.



4. **Approved**

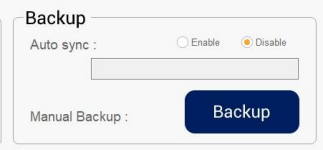
After data have been approved, the approved data will be shown in the “Approved data”. It is possible to **‘Export’** approved data.

21 CFR part 11

Setting

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

1) Backup



① Click the Auto sync **'Enable'** and set a path to back up data automatically. We recommend to use an external hard drive for "Auto sync" option.

② Click the Manual **'Backup'** button to back up data manually.

2) Restore

The Restore function can be useful to restore data in the unlikely events of hard drive failure or system failure.

Cleaning and Maintenance

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

ADAMII™ LS does not need regular maintenance. To troubleshoot problems with ADAMII™ LS, contact technical support.

⚠ **IMPORTANT! Never disassemble or service ADAMII™ LS by yourself.**

Unauthorized repairs may damage ADAMII™ LS 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 ADAMII™ LS.

⚠ **IMPORTANT! Avoid exposing ADAMII™ LS to UV light.**

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

Troubleshooting

Installation	
ADAMII™ LS does not power on	• Check on/off switch on back 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
Camera preview	
Camera preview fail	• Check connection between instrument and PC • Reboot ADAMII™ LS software • Reboot the instrument or PC • Request technical support
Focus varies from cell to cell within a frame	• Make the settle time longer after load your sample
Fluorescence is too weak	• Increase the camera settings • Try changing the staining protocol
Fluorescence is too bright	• Lower the camera settings • Try changing the staining protocol
Fluorescence doesn't show anything	• Make sure the sample is properly stained. • Check fluorescence channel type • Increase the camera settings • If it is dark even with correct samples and high camera settings, request technical support
Measure	
After starting the measurement, the Bright channel is taken twice	• Normal operation. Allow time for the sample to stabilize.
The slide wall is photographed	• Put the slide back in. Push the slide all the way in. Check the direction of the slide • Check the slide type (1ch or 2ch slide) • Request technical support

Warranty

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

If any defects occur in ADAMII™ LS, 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, ADAMII™ LS 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 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, please 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 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.

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
	The LAN Port symbol identifies the communication between the medical device and the only external computer.
	Catalogue number/Reference number
	Serial number
	Manufacturer
	Electrical rating
	Use by YYYY-MM-DD or YYYY-MM

	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
	Keep dry Keep away from rain
	Fragile, handle with care
	This way up
	General symbol for recover/recyclable
	Team lift
	Temperature limitation
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

ADAMII™ LS	
Lens	10 x
Light source	Bright field, UV, Blue, Green LED
Analysis time	App. 30 sec ~ 4 min 30 sec * (1 ch slide) App. 45 sec ~ 6 min 45 sec * (2 ch slide)
Loading volume	25 µL
Measuring volume	≤ 7.8 µL (1 ch slide) ≤ 5.7 µL (2 ch slide)
Measurement range	5 x 10^4 ~ 5 x 10^6 cells/mL
Cell size range	3 ~ 60 µm
Electronic input	12VDC, 5.0A
Operating power	100 – 240 V, 1.5 A, 50/60 Hz
Dimension	300 mm (W) x 420 mm (D) x 370 mm (H)
Weight	19.3 kg
*Depends on assay or frame.	
Operation	
Temperature	5℃ ≤ Temperature ≤ 40℃
Humidity	20% ≤ Humidity ≤ 80%
Altitude	Altitude ≤ 2,000 m
Transportation & storage environment condition	
Temperature	- 30℃ ≤ Temperature ≤ 60℃
Humidity	10% ≤ Humidity ≤ 90%
Assay reagents	
Storage	2 ~ 8 ℃
Expiration date	1 year from date of manufacture
Assay slide	
Storage	15 ~ 30 ℃
Expiration date	2 years from date of manufacture

Ordering information

Cat. No.	Product	Contents
ADAMII-LS	Fluorescence cell analyzer	• Main device • Lab top PC with operating SW installed • Instruction manual
ALAD-100	Cell viability reagent	• Acridine orange (AO) & 4',6-diamidino-2-phenylindole (DAPI) stain 0.5 mL x 2 tubes (100 Tests)
ALPI-100	PI cell cycle reagent	• Propidium Iodide (PI) stain: 1.25 mL x 2 tubes (100 Tests)
ALAP-100	Apoptosis detection kit	• AnnexinV-PE stain 0.5 mL x 1 tube (100 Tests) • DAPI solution: 125 µL x 1 tube (100 Tests) • AnnexinV binding buffer 10 mL x 1 tube (100 tests)
A2AS-051	ADAMII Assay slide 1ch	• 1 ch x 50 slides/box
A2AS-052	ADAMII Assay slide 2ch	• 2 ch x 50 slides/box
ADAMII-LS 21 CFR Part 11	ADAMII-LS 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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ADAMII LS

NESMU-A2LS-001E (V.0.6)

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