CE

ExTransfection™

Electroporation System

User manual

NanoEntek

ExTransfection™ 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.

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Table of Contents

Introduction

Summary

ExTransfection™ Electroporation System is an innovative, efficient, and bench-top electroporation device that enables efficient transfection of cell lines, primary cells, stem cells, and hematopoietic cells.

ExTransfection™ requires ExTransfection™ kit which includes disposable pipette tips and pre-made buffer solutions.

ExTransfection™ transfects from 1×10^4 to 5×10^6 cells per electroporation and offers 2 sample volumes; 10 µL or 100 µL.

ExTransfection™ delivers nucleic acids, proteins, and siRNAs to mammalian cells while maintaining high viability of cells.

ExTransfection™ provides 18-well and 24-well optimization method to help users to optimize transfection protocols easily and quickly. Once an optimal protocol has been identified, ExTransfection™ allows users to save their own protocols.

ExTransfection™ also provides a library of 320 protocols that covers various commonly used cell lines and primary cells. Furthermore, ExTransfection™ allows users to modify protocols in the library and save modified version.

More information about the parts and components of the system can be found in the "Product Components" on page 7.

Introduction

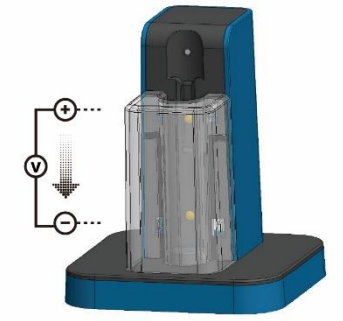
Key Features

- **High-Efficiency** - Delivers intensive and even electrical pulses without unexpected loss
- **Superior Viability** - Maintains high cell viability throughout the process
- **Applicable** - Compatible with various cell types, including mammalian, primary, stem, and blood cells
- **Consistent** - Ensures reproducible results under identical conditions
- **Usability** - Supports 10 µL and 100 µL sample volumes for different experimental needs
- **Optimized Protocol** - Optimized Protocol – The protocol library includes more than 300 cell-specific protocols

System Principle

Unlike traditional cuvette-based electroporation methods, ExTransfection™ Electroporation System utilizes a proprietary electroporation reaction chamber, ExTransfection™ tip, to deliver a strong electric field to a biological sample. The system is designed to minimize surface area of two electrodes while increasing distance between them, so that cells will be exposed to a uniform electric field.

This uniform electric field minimize damages on cells while minimizing pH variation, ionization of buffer, and heat production. This electroporation technology improves transfection efficiency and cell viability.



Product Components

ExTransfection™ Electroporation System

ExTransfection™ consists of the following components.

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

• Contents

ExTransfection™ Device and accessories		
EXT1000	ExTransfection, Electroporation System	Main device, Pipette, Pipette station
EXT1000P	ExTransfection Pipette	Pipette (1 ea)
EXT1000PS	ExTransfection Pipette Station	Pipette Station (1 ea)

ExTransfection™ Kit

ExTransfection™ kits should be used together with ExTransfection™ Electroporation System (Refer to "Ordering information" in page 68).

• Contents

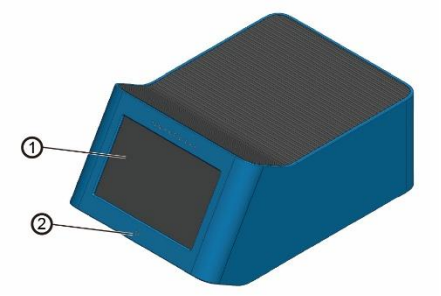
Consumables		
EXT1025K	ExTransfection™ 10μL Kit	Resuspension buffer R (1 ml)
		Resuspension buffer T (1 ml)
		Electrolytic buffer E (75 ml)
		Disposable tip (10 μl, 25 tips)
		Disposable tube (5 ea)
EXT10025K	ExTransfection™ 100μL Kit	Resuspension buffer R (10 ml)
		Resuspension buffer T (10 ml)
		Electrolytic buffer E2 (75 ml)
		Disposable tip (100 μl, 25 tips)
		Disposable tube (5 ea)
EXT1096K	ExTransfection™ 10μL Kit	Resuspension buffer R (1 ml, 3 ea)
		Resuspension buffer T (1 ml, 3 ea)
		Electrolytic buffer E (150 ml, 2 ea)
		Disposable tip (10 μl, 96 tips)
		Disposable tube (20 ea)
EXT10096K	ExTransfection™ 100μL Kit	Resuspension buffer R (30 ml)
		Resuspension buffer T (30 ml)
		Electrolytic buffer E2 (150 ml × 2 ea)
		Disposable tip (100 μl, 96 tips)
		Disposable tube (20 ea)
EXT50T	ExTransfection™ Tube	Disposable tube (5 ea, 10 packs)

Product Description

Device

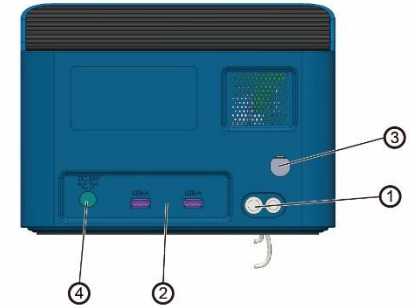
ExTransfection™ devices are designed to transfect mammalian cells, especially primary cells, stem cells, and hematopoietic cells, utilizing the pipette tip as an electroporation reaction chamber.
It has a built-in 18-well and 24-well optimization protocol and also allows users to store custom protocols

Front view



① Touch-enabled user interface screen
② Power switch

Rear view

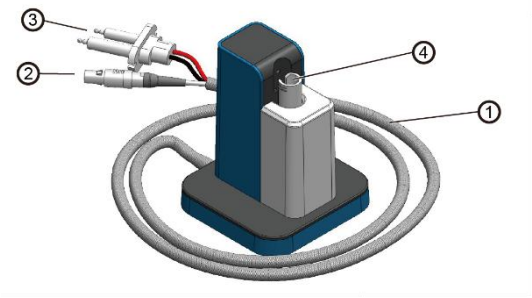


① High voltage ports to connect with ExTransfection™ pipette station
② USB port panel for an USB port for portable USB memory device. Also used for QC and Service.
③ Sensor port to connect with ExTransfection™ pipette station
④ DC inlet

Product Description

Pipette station

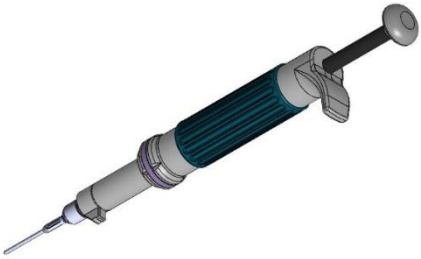
ExTransfection™ pipette station supplies high-voltage power to ExTransfection™ pipette during electroporation. It also monitors any electric shocks during electroporation as a safety measure. In addition, ExTransfection™ pipette station works as a stable holder for ExTransfection™ pipette during electroporation.



① Connector cable
② Sensor connector
③ High voltage connector
④ Area to insert ExTransfection™ tube

Pipette

ExTransfection™ pipette is a positive displacement pipette which helps handling of small volumes of solutions containing cells, DNA or siRNA. ExTransfection™ pipette is pre-calibrated during manufacturing.
ExTransfection™ pipettes are designed to be used with proprietary ExTransfection™ tips that come with ExTransfection™ kits. Using other types of tips may result in poor performance.



Product Description

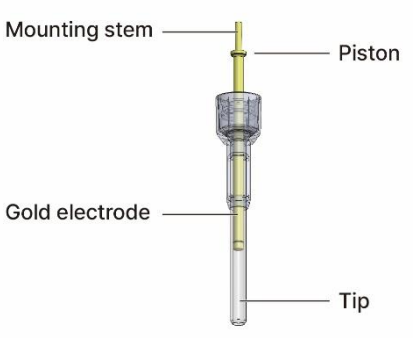
Tip

ExTransfection™ tips are disposable tips that consist of a tip and piston, designed for use with ExTransfection™ pipette. ExTransfection™ tips contains gold-plated electrode to generate electric field within tips.
ExTransfection™ tips are included in ExTransfection™ kits.
ExTransfection™ tips are available in two formats; 10 µL and 100 µL (Refer to page 68 for ordering information).

ExTransfection™ tips can be used up to 2 times. Repeated use may cause oxide formation within tips which can impair generation of homogeneous electric field.

⚠ ***CAUTION***
To prevent bubbles from forming, ExTransfection™ tips should be fully loaded with the prepared mixture once to completely wet the inside before use.

Tip specifications:
Material: Polypropylene
Capacity: 10 µL or 100 µL



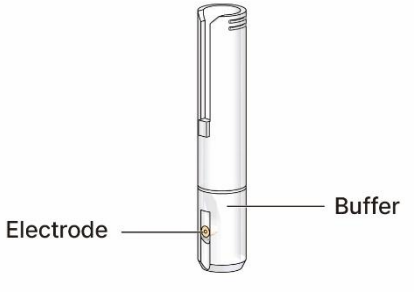
Product Description

Tube

ExTransfection™ tube is a semi-reusable tube that needs to be placed in ExTransfection™ pipette station and serves as a holder for electrolytic buffer during electroporation. ExTransfection™ tube contains an electrode near the bottom.
ExTransfection™ tubes are provided with ExTransfection™ kits and can also be purchased separately (refer to page 68.
ExTransfection™ tube can be used up to 10 times but due to contamination risks, it is recommended to replace ExTransfection™ tube at least every 10 uses.

Additionally, if cell types or plasmid (DNA or siRNA) are changed, it is recommended to replace ExTransfection™ tube.

Tube Specifications:
Material: Polystyrene
Capacity: 2.5–4 mL



Buffer Kits

ExTransfection™ kits include buffers that will be required to operate ExTransfection™. ExTransfection™ kit consists of two components (tip/tube kit and buffer kit), and is available in two sample volumes, 10 µL or 100 µL. ExTransfection™ kits are shipped at room temperature. After receiving the kit, it is recommended to store the buffers at 4°C, and the tips and tubes at room temperature.

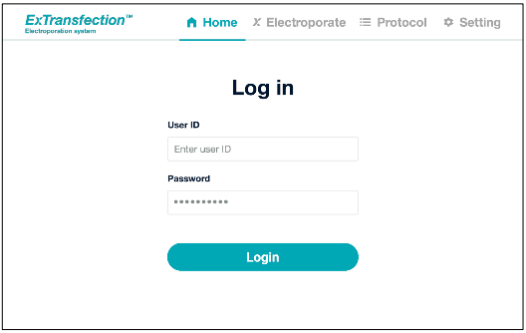
User Interface Description

Overview

Tab	Function
Home	• Login with user ID and PW • When a protocol is saved, the current user ID will be included in the saved protocol. • When Master ID was used, Master can manage users to create new user or modify current users.
Electroporate	• Run electroporation • Choose a protocol from the library • Run optimization • Calculator App to help sample preparations
Protocol	• Review saved protocols • Add new protocol or edit an existing protocol • Manage protocols
Setting	• User management • Self-diagnostics program • Set time and date • Update software and firmware (USB drive required) • Review and export logs

Tab

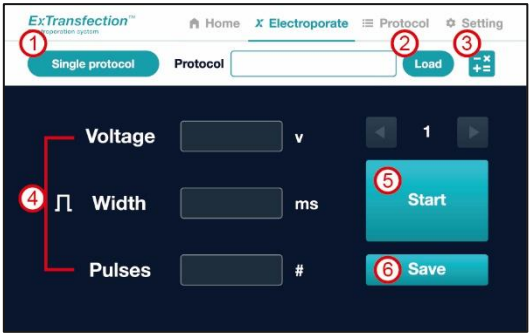
■ Home Tab



To use ExTransfection™, one needs to log in with a user ID and PW. To make a new user ID, one needs to log in with Master ID. The default Master ID and PW are “master” and “master”. Please make sure to change the password for Master ID.

Tab

■ Electroporate Tab



① **Protocol Type (Single protocol / Optimization)**
Click the button to switch between “Single protocol” and “Optimization”.
If one runs electroporation with samples, choose “Single protocol”. If one runs optimization, choose “Optimization”.

② **Load**
Load a protocol from the lists of saved protocol or a preinstalled library of protocols.

③ **Calculator**
Calculate how to prepare samples such as calculating the volume of media to add or the number of cells to start with.

④ **Electroporation Parameters**
If one loads a protocol, these parameters will be populated from the saved values. Otherwise, one can enter manually.

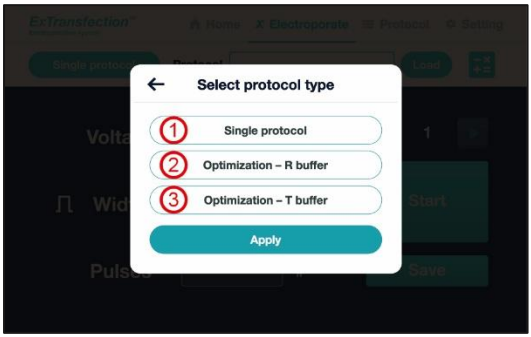
⑤ **‘Start’ button**
Click “Start” button to run electroporation

⑥ **‘Save’ button**
To save the current sets of parameters

User Interface Description

Tab

Select protocol type



When one clicks 

, a popup window will appear and ask user to choose Single protocol or one of Optimization protocols.

① **Single protocol**

One can use this option to use a saved protocol or to enter electroporation parameters manually.

② **Optimization – R buffer**

Once can use this option to run optimization using R buffer. This option works for most cell types including all cell lines.

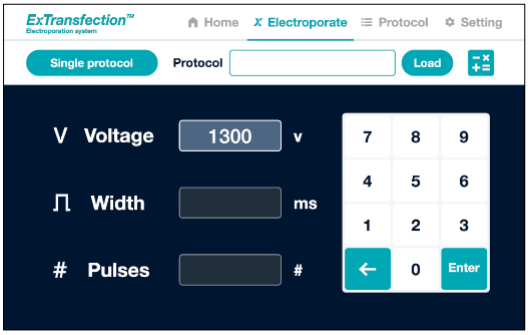
③ **Optimization – T buffer**

One can use this option to run optimization using T buffer. This option can be used for cells that generally require high voltage, such as cells isolated from blood.

User Interface Description

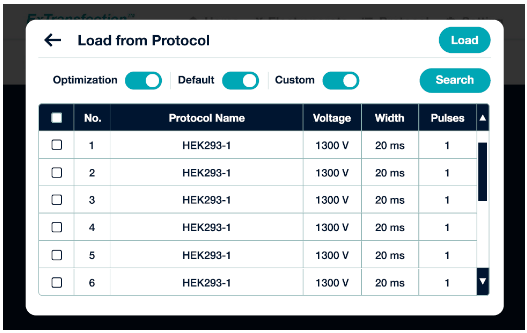
Tab

Enter parameter values



If one clicks a space to enter a value, numeric keypad will appear. Repeat to enter values for all 3 electroporation parameters; Voltage, Width, Pulse.
If the entered value is out of the allowable ranges, an error message will appear.

Load from Single Protocol

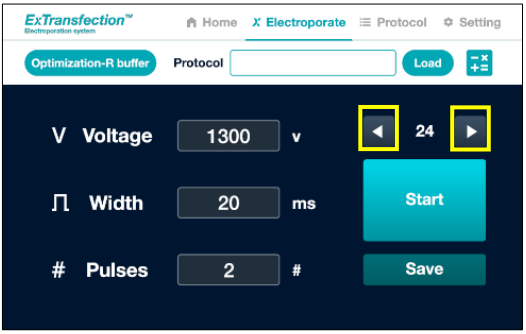


When clicking “Load” button, the lists of saved protocol will appear and one can choose a protocol. By choosing “Optimization”, “Default” or “Custom”, one can filter out corresponding sets of protocols. By choosing “Search” button, one can search a protocol based on the protocol names.
(Refer to page 33 for Database information.)

User Interface Description

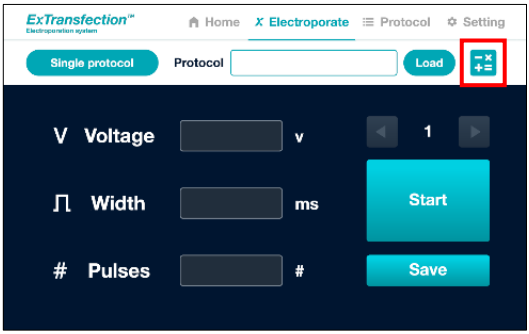
Tab

Built-in optimization protocol



When Optimization is selected as the protocol type, optimization can be performed using the built-in optimizatio protocol. Depending on the buffer selection, this protocol includes either 24 or 18 different electroporation parameters. The arrows located above the Start button allow easy navigation between protocols.

Sample volume calculator

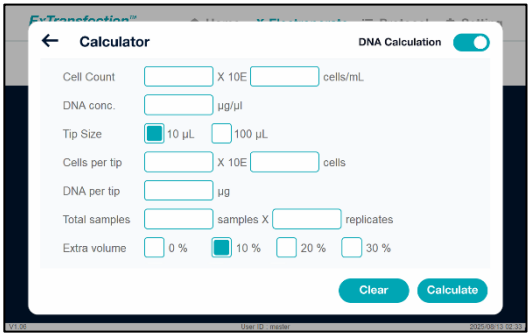


One can use Calculator App to find out how to prepare cells and DNA/RNAs. First, enter the concentrations of both cell solution and DNA/RNA solution. Then click “Calculate”. The App will show how many cells and how much DNA/RNA to be added per each pipetting.

Tab

Since the number of cells harvested may vary from user to user, the App does not require you to enter the total cell volume. Instead, calculations are based on the concentration of the cell solution. This way, the App can provide the exact number of cells needed per pipetting, regardless of the total harvested volume.

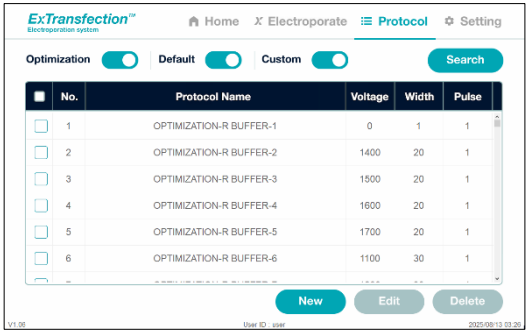
By choosing extra volume (between 0 ~ 30%), one can prepare slightly more sample than required to compensate for pipetting loss. The App will then calculate how many samples and replicates can be run with the prepared samples.



If one prefer to use a fixed amount of 1 µL of DNA/RNA/RNP per well, please turn off the DNA Calculation toggle before use.

Tab

■ Protocol Tab



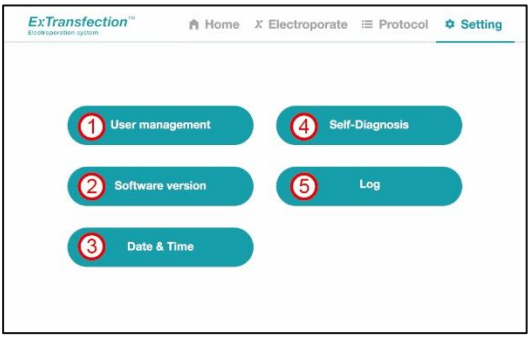
In the “Protocol Tab”, the lists of saved protocols are provided including the protocols to be used for optimization, the protocols saved by users, and a library of 320 protocols for commonly used cell lines and primary cells. The library of 320 protocol is under the category of “Default”.

NOTE: One can use filters (Optimization, Default, Custom) to show only those protocols in the selected category.
NOTE: One can search a protocol based on the name by using “Search” button. (refer to page 35)

User Interface Description

Tab

■ Setting Tab



① **User management**
To manage user accounts

② **Software version**
To check the version of current software and firmware, and if needed, to run software updates using a USB drive.

③ **Date & Time**
To set time and date

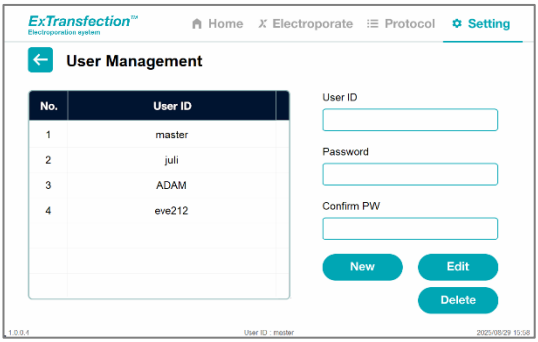
④ **Self-Diagnosis**
To run self-diagnosis to check and verify the health status of the instrument.

⑤ **Log**
To review and export the logs saved in the instrument.

User Interface Description

Tab

User Management



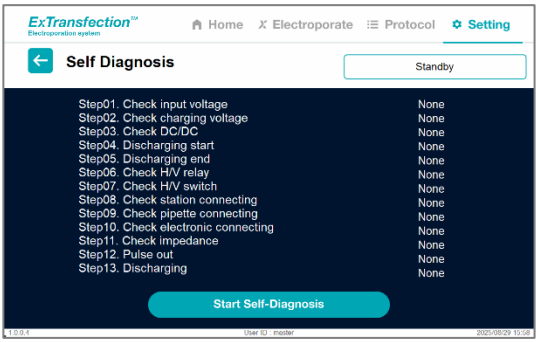
When one has logged in using “Master” ID, “User Management” in the “Setting” tab will be activated. Using this management function, one can add new user or delete a user. When one has logged in using a non-Master ID, one can only change one’s own password. Both ID and PW should be 30 characters or less.

※ If one forgot the password for the “Master” ID, please contact technical support.

User Interface Description

Tab

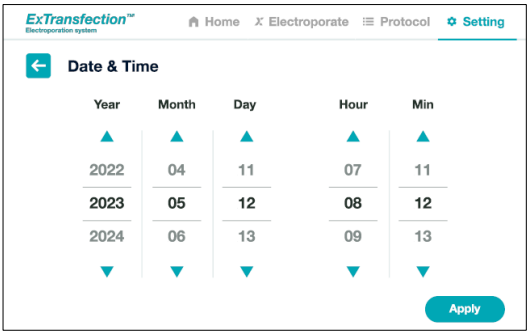
Self-Diagnosis



"Self-Diagnosis" option allows ones to check the health status of the instrument including connection status with pipette station and working condition of high voltage management system.
Before using "Self-Diagnosis", one has to fill the tube and pipette tip with buffer. For 10 µL tip, please use E buffer and 100µL tip, please use E2 buffer.

※ If there is error during self-diagnosis, please stop using the instrument and contact support immediately.

Date & Time

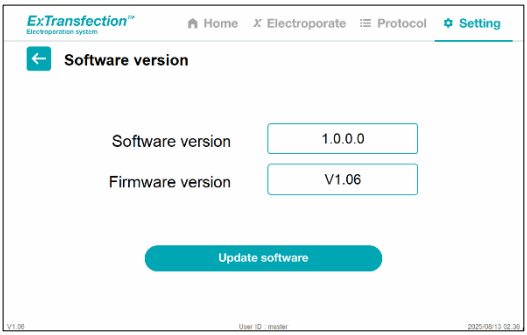


One can change time and date manually.
Only Master ID can access this menu.

User Interface Description

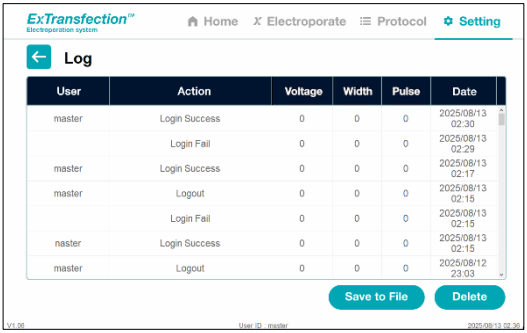
Tab

Software version



One can check the current versions of software and firmware. When new software becomes available, one can update by using a USB memory drive.
When a USB drive containing update files is connected to the instruments, the “Update software” button will be activated. Click the “Update software” button to update software or firmware. After completing updates, the software will automatically reboot.

Log history



One can review the logs of user activities including log-ins and running experiments. One can export the logs into a file through USB. One can also delete all logs.

※ Only Master ID can have access to Log history.

※ Deleting or modifying selected logs is not allowed.

Installation

Environmental Requirements

Inspection Upon Receiving the Device
Carefully inspect the equipment for damage in transit. If one notice any damage, report it to the carrier, or dealer, or NanoEntek immediately. If one needs further assistance, please contact NanoEntek.

NOTE: If one has a plan to move or ship ExTransfection™, please keep carton case and other packing materials.

For best performance, please review the following recommendations to set up ExTransfection™

① ExTransfection™ allows DC power of 24V adaptor. Before one connect power cord to ExTransfection™, please make sure that the power switch is in off position.

② Place ExTransfection™ on a flat surface, such as a laboratory bench. ExTransfection™ can also be placed in a biosafety cabinet or a cell culture hood.

③ During operation, ExTransfection™ can be hot. For one's safety, please make sure that there are enough spaces (at least 10 cm or 4 inches) around ExTransfection™. Also, please make sure that power switch and power plug are easily accessible so that ExTransfection™ can be turned off quickly if needed.

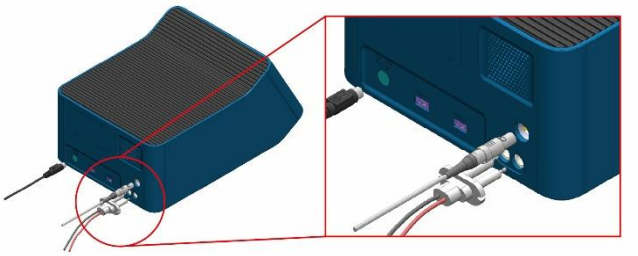
④ ExTransfection™ pipette station needs to be put near ExTransfection™.

⑤ Connect ExTransfection™ pipette station with ExTransfection™ using high voltage and sensor cables for which connecting ports are in the back of ExTransfection™.

Installation

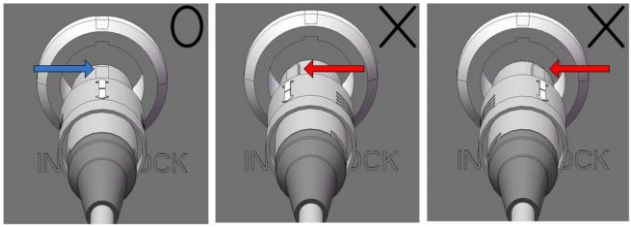
⚠ ***CAUTION***

When connecting or disconnecting the connector, always hold the plug, not the cable.



⚠ ***CAUTION***

When connecting the sensor cables to the interlock code, make sure to align the arrow markings properly



⑥ To turn it on, press the power switch in the front. Once ExTransfection™ is turned on, the display will show a welcome screen.

⑦ ExTransfection™ is operated by using the touchscreen which can be controlled with either a fingertip or a touchscreen-compatible stylus.

General guidelines

General guidelines

ExTransfection™ should be used with ExTransfection™ kits. ExTransfection™ is not compatible with other transfection or electrophoresis kits. Ordering information for ExTransfection™ kits can be found on page 68.

It is strongly recommended to optimize electroporation protocols at the beginning of using the instrument or when using a new cell type. Two optimization protocols are provided to help users to run optimization easily.
Optimization using R buffer can be used for most of cell types and it includes 24 different electroporation protocols.
Optimization using T buffer can be used for primary cells or PBMCs, and it includes 18 different electroporation protocols.

One can choose one of three protocol types to run electroporation as shown in the page 14.

General guidelines

Preparation before use

When performing electrophoresis, proper personal protective equipment (PPE) should be worn. PPE should include gloves, lab coats, and safety glasses.
Please make sure that ExTransfection™ tip is properly attached to ExTransfection™ pipette, and ExTransfection™ pipette is properly inserted to ExTransfection™ tube within ExTransfection™ pipette station.

Compatibility and Precautions

ExTransfection™ is compatible with most mammalian cells, primary cells and stem cells.
It is important to use high-quality DNA and siRNAs to increase transfection efficiency.
ExTransfection™ tips and ExTransfection™ tubes should be discarded after 2 uses and 10 uses, respectively. If plasmid DNA/siRNA or cell type is changed, it is recommended to replace ExTransfection™ tube and buffer.

General guidelines

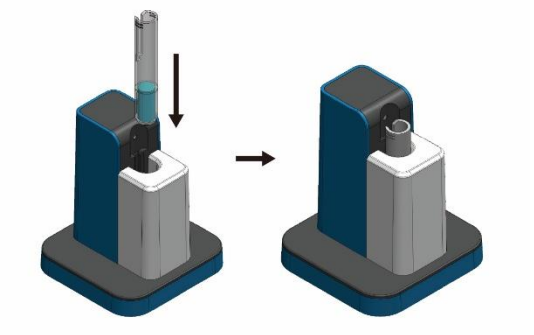
Pipette station & Tube

1. Make sure that ExTransfection™ pipette station is properly connected to ExTransfection™ device. (Refer to page 24).

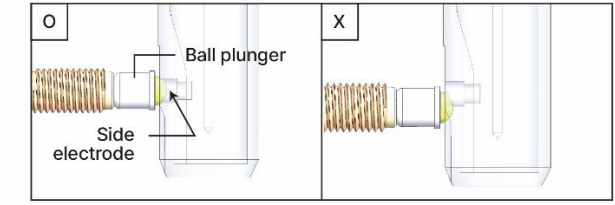
2. Fill 3mL of buffer to ExTransfection™ tube. Use Buffer E for 10µL tips and Buffer E2 for 100µL tips.

   NOTE: The tube has a marking for a 3 mL capacity, allowing convenient filling of the buffer.
   NOTE: Make sure the electrode on the side of ExTransfection™ tube is fully immersed in the buffer.

3. Insert ExTransfection™ tube into ExTransfection™ Pipette Station until one hears “click” sound.



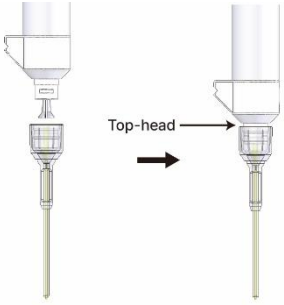
NOTE: Make sure that the electrode on the side of ExTransfection™ tube makes full contact with the ball plunger in ExTransfection™ tube. (Refer to the diagram below.)



General guidelines

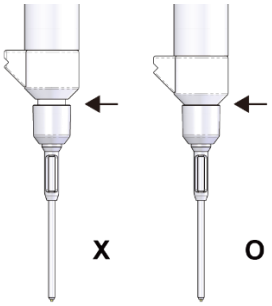
Pipette & Tip

1. To attach an ExTransfection™ tip, push the plunger of ExTransfection™ pipette to the second stop position to expose clamp at the end of ExTransfection™ pipette.
2. While holding the plunger at the second stop, insert the clamp into an ExTransfection™ tip. (Refer to the diagram below)



3. Slowly release the plunger while continuing to apply downward pressure to the tip so that the tip is in full contact with the pipette.

NOTE: ExTransfection™ pipettes and tips must be connected tightly to ensure stable connection with electrode in the tip (Refer to the diagram below).



4. To aspirate solution (sample consisting of cells and payload), press the plunger of ExTransfection™ pipette to the first stop, immerse ExTransfection™ tip into a solution, slowly release the plunger.

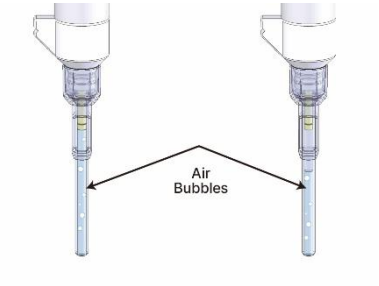
NOTE: Make sure that there is no air bubbles in the tip. If air bubbles are trapped within the tip, an arc can form during electroporation, and transfection may not work.

General guidelines

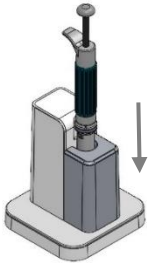
Pipette & Tip

⚠ ***CAUTION***

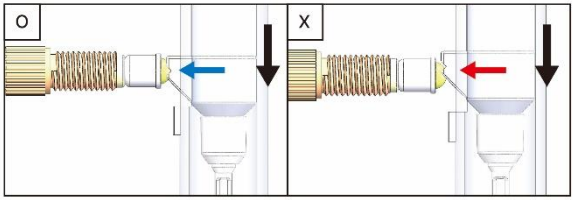
To prevent bubbles from forming, ExTransfection™ tips should be fully loaded with the prepared mixture once to completely wet the inside before use.



5. To insert ExTransfection™ pipette into ExTransfection™ pipette station, hold ExTransfection™ pipette vertically, and slowly insert ExTransfection™ pipette into ExTransfection™ tube until one hears “click” sound.



NOTE: Make sure that the electrode on the side of ExTransfection™ pipette makes full contact with the ball plunger in ExTransfection™ pipette station.



Electroporate

Input values limit

ExTransfection™ has limits on the parameters as shown below. If input value is out of these ranges, an error will be displayed.

Input range
Input voltage range: 500–2,500 V
Input pulse width range: 1–100 milliseconds
Input pulse count range: 1–10

Over power limit
To prevent device damage, a limit has been set on the final power value. An error occurs if the threshold exceeds 144.

$$144 = (\frac{Voltage}{1000})^4 \times Width^2 \times (\frac{Pulses}{100})$$

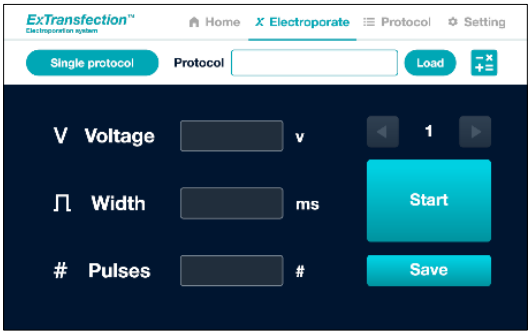
Ex) 2500 V, 20ms, 1 pulse > 144 ➔ ERROR

NOTE: If the input value exceeds the limit, an error message will be displayed and the value will revert to the previous value.

Protocol Input

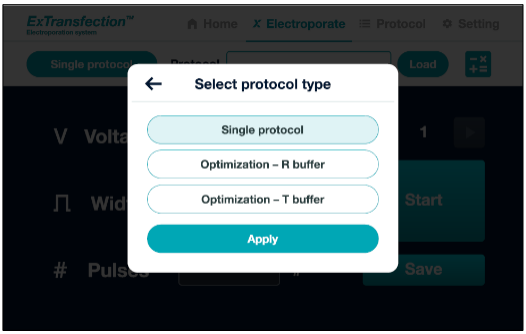
When one knows electroporation parameters, please follow the steps below to run electroporation.

1. First, make sure that pipette station is properly connected to the instrument. Turn on the instrument and log in with ID and PW. Choose “Electroporate” tab.



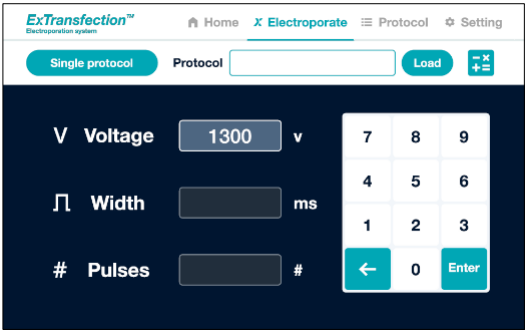
Protocol Input

2. Make sure that “Single protocol” has been chosen. If not, choose “Single protocol” in the ‘Select Protocol Type’



3. Enter values in each variable; Voltage (the voltage of electric pulses to apply), Width (the width of electric pulses to apply), and # Pulses (the number of pulses to apply). After entering all three variables, press “Start” button to start electroporation.

NOTE: If the input value exceeds the limit, an error message will be displayed and the value will revert to the previous value.

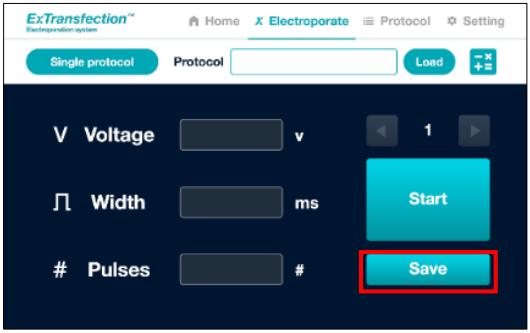


4. If cells and DNA have not been prepared, please see page 39~43 to prepare samples.

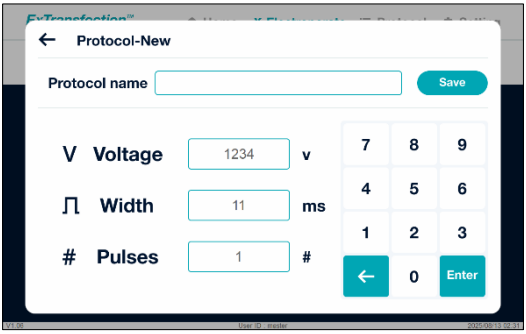
Protocol Save

One can save the current parameters as a protocol.

1. Click “Save” button to save the current parameters (Voltage, Width and Pulse).



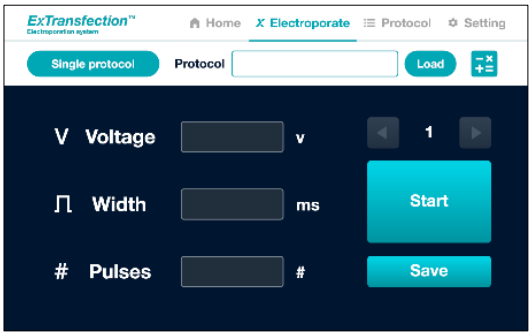
2. Enter a protocol name to save the parameters. The protocol will be saved in the protocol library and can be loaded later.



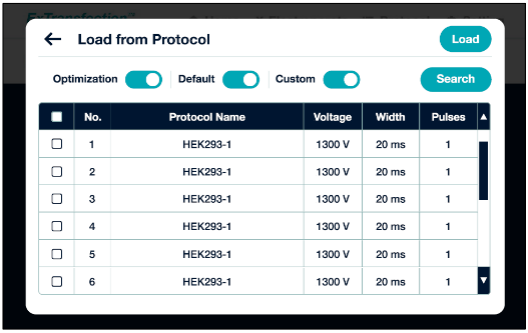
Database Protocol

When one uses parameters from one of saved protocols, please follow the steps below to run electroporation.

1. First, make sure that pipette station is properly connected to the instrument. Turn on the instrument and log in with ID and PW. Choose “Electroporate” tab.



2. Clock “Load” button to open protocol library window.



3. Choose a protocol from the lists or use “Search” option to find a protocol. One can also use filters (Optimization, Default, and Custom) to hide or show protocols in each category.

Electroporate

Database Protocol

- **Optimization**
  There are 24 protocols for “Optimization using R buffer” and 18 protocols for “Optimization using T buffer”.
- **Default**
  There are 320 protocols which have been tested by NanoEntek with various cell types including primary cells and PBMCs.
- **Custom**
  In this category, those protocols saved by users will be listed.

  NOTE: One can only see those protocols saved by oneself. One will not be able to see the protocols saved by other users.

4. Once choosing a protocol, click “Load” button to import parameters. Only one protocol can be selected.

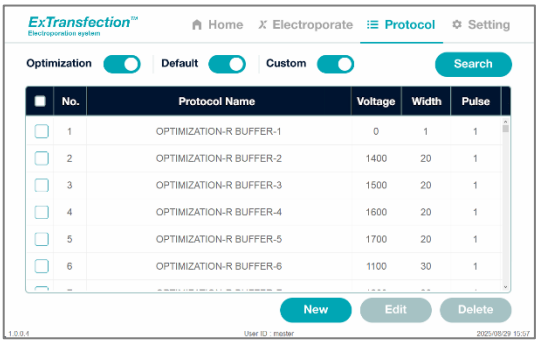
5. If cells and DNA have not been prepared, please see page 39~43 to prepare samples.

Protocol

Database Protocol

You can review the list of saved protocols and create up to 200 new protocols.

<Protocol tab>



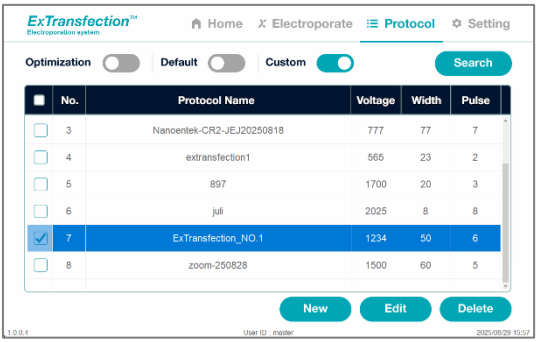
1. One can also use filters (Optimization, Default, and Custom) to hide or show protocols in each category.

   - **Optimization**
     There are 24 protocols for “Optimization using R buffer” and 18 protocols for “Optimization using T buffer”.
   - **Default**
     There are 320 protocols which have been tested by NanoEntek with various cell types including primary cells and PBMCs.
   - **Custom**
     In this category, those protocols saved by users will be listed.

     NOTE: One can only see those protocols saved by oneself. One will not be able to see the protocols saved by other users.

2. One can use “Search” option to find protocols by their names.

Database Protocol



3. You can create a new protocol using the “New” button (up to 200 protocols).
   Using the “Edit” button, you can modify an existing protocol and save it as a new protocol.
   The “Delete” button allows you to delete protocols. However, you can only edit or delete protocols that you have saved yourself; optimization protocols and the 320 default protocols cannot be deleted.

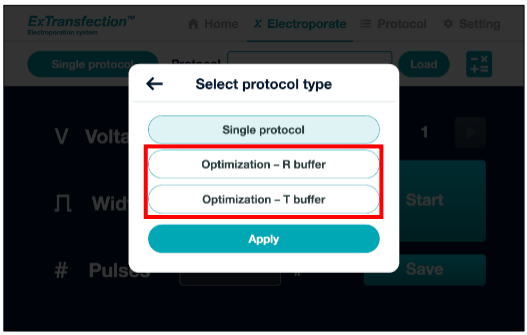
Optimization Protocol

It is an important and often times necessary step to find optimal electroporation conditions for each cell types.
One can use the pre-loaded optimization protocols to find best electroporation conditions.
Within the instrument, there are two sets of optimization protocols and these protocols can't be modified.
For detail sets of parameters for these optimization protocols, please see page 47.

1. First, make sure that pipette station is properly connected to the instrument. Turn on the instrument and log in with ID and PW. Choose "Electroporate" tab.

2. Make sure that one of Optimization has been chosen as a protocol type. If not, choose either "Optimization -R buffer" or "Optimization – T buffer" in the 'Select Protocol Type'

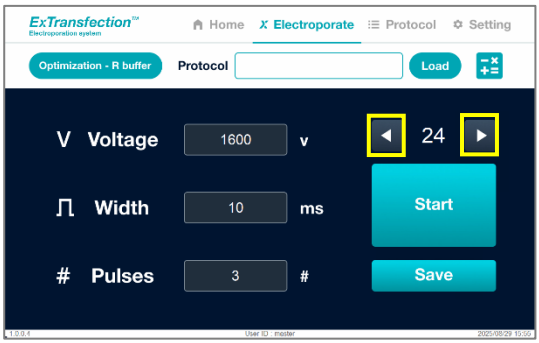
※ Optimization using R buffer works for most cell types including all cell lines and includes 24 different protocols. Optimization using T buffer is suitable for cells isolated from blood or cells that generally require high voltage, and includes a total of 18 different protocols.



Optimization Protocol

3. Depending on the optimization type, either 24 or 18 sets of protocols will be loaded. One can move between protocols by using the arrow buttons.

   NOTE: The detailed electroporation parameters in the optimization protocols can be found in the "Protocol" tab.



4. Proceed with cell preparation (Refer to pages 39-43), DNA preparation, and setting up the ExTransfection™ pipette station for electroporation (Refer to page 27).

Cell Preparation

Cell solution

Cell Preparation Process

※ For convenience, you can use the device's calculator function.

1. Prepare following solutions
   - Cell culture media with serum (it may contain other supplements including antibiotics) pre-warmed up to 37°C.
   - Cell culture media without antibiotics (it may contain serum and other supplements, but no antibiotics) pre-warmed up at 37°C.
   - Resuspension buffer R for a protocol using voltage less than 1900V or resuspension buffer T for a protocol using voltage 1900 V or higher, pre-warmed up at 37°C.
   - Dissociation agent (e.g., Trypsin) pre-warmed up at 37°C
   - PBS without Ca^{2+} and Mg^{2+}
   - Well plates
   - 1.5 ml micro tube
   - Centrifuge tube for cell collect

2. Prepare cell solutions. Detailed concentrations and amounts of cells for different formats of plates can be found in **Table** 1 (Page 46).

3. For adherent cells, suggested protocols are as below;
   - Aspirate cell culture media from a cell culture vessel (flask or petri-dish)
   - Wash cell culture vessel with PBS
   - After aspirating PBS, treat cells with dissociation agent.
   - When cells are detached, collect cells into a centrifuge tube.
   - Take an aliquot of cells for cell counting and centrifuge cell-containing tube at 100~400G for 5 minutes.
   - Count number of cells. (Using automated cell counting equipment such as EVE™ Plus and ADAM™ MC Plus, it is fast and convenient)
   - Remove supernatant, and add PBS without Ca^{2+} and Mg^{2+} and re-suspend cell pellet
   - Transfer the required amount of cells into a new 15 mL tube, and centrifuge again at 100-400G for 5 minutes.

Cell Preparation

Cell solution

- Remove PBS, and add resuspension buffer to adjust the cell concentration at **1x10^7** cells/mL
  - For a protocol using voltage less than 1900 V, use resuspension buffer R
  - For a protocol using voltage 1900V or higher, use resuspension buffer T

CAUTION: *Do not store cell solution at room temperature for more than 15 minutes. Keeping cells in solution for long may decrease cell viability and transfection efficiency.*

*NOTE**:** Cell concentration may be adjusted to match the number of cells required.*

3-1. For suspension cells, suggested protocols are as below;

- Collect cells into a centrifuge tube.
- Make an aliquot of cells for cell counting.
- Count number of cells. (Using automated cell counting equipment such as EVE™ Plus and ADAM™ MC Plus, it is fast and convenient)
- Remove media, and add PBS without Ca^{2+} and Mg^{2+} and re-suspend cell pellet
- Transfer the required amount of cells into a new centrifuge tube, and centrifuge again at 100-400G for 5 minutes.
- Remove PBS, and add resuspension buffer to adjust the cell concentration at **2x10^7** cells/mL
  - For a protocol using voltage less than 1900V, use resuspension buffer R
  - For a protocol using voltage 1900V or higher, use resuspension buffer T
- For example, to run a 24-well based optimization protocol, at least 300 µL of cell suspension is required.

CAUTION*: Do not store cell solution at room temperature for more than 15 minutes. Keeping cells in solution for too long may decrease cell viability and transfection efficiency.*

NOTE: Cell concentration may be adjusted to match the number of cells required.

Cell Preparation

Cell solution

4. Prepare plate before electroporation.
   - Fill wells with cell culture media without antibiotics. For example, fill 500 µL of media per each well in 24-well plate.
   - Refer to the table 1 for suggested media volume for other types of vessels.

※ It is important to avoid bubbles within tips which may cause arcs during electroporation. Therefore, it is important to prepare enough volume of samples so that tip can be completely submerged through the last pipetting.

※ When loading samples into a tip, make sure that the sample solution has been well mixed.

Nucleic acid preparation

DNA quality and amount

General guide to prepare DNA for electroporation

※ For convenience, you can use the device's calculator function

It is important to use high-quality DNA at a right concentration for high transfection efficiency.
The purity of the DNA should be measured by the A260/280 ratio and it needs to be at least 1.8 or above. If this ratio is low, DNA may contain impurities.
ExTransfection™ has been tested with purified DNA of 4–7 kb and can be used with DNAs of up to approximately 20 kb. However, DNAs of over 20 kb may reduce transfection efficiency.

⚠ **Important NOTE**
Never enrich DNA using ethanol precipitation method. DNA enriched through ethanol precipitation can result in reduced transfection efficiency and reduced cell viability due to salt contamination.

⚠ **Important NOTE**
Purified DNA can be resuspended in deionized water or Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at a concentration of 1–5 µg/µL. (However, the optimal concentration may vary depending on the cell types.)
The amount of DNA solution should be less than 10% of cell solution to avoid drastic changes in ion concentrations or osmotic pressure.

GFP Control
To evaluate transfection efficiency using fluorescence microscopy, it is recommended to use plasmids expressing green fluorescent proteins (GFPs). For best results, choose a vector with the following characteristics:

- Include CMV (Cytomegalovirus) promoters that are strongly expressed in a variety of mammalian cells
- Include SV40 polyA signaling (appropriate processing of the 3' end of GFP mRNA)
- Has antibiotic selection markers
- Include pUC replication starting point (can be amplified within E. coli)

siRNA quality and amount

General guide to prepare siRNA for electroporation

Quality and concentration of siRNA have significant impacts on transfection efficiency. Therefore, high-quality siRNAs are recommended to use for electroporation.
Recommended siRNA concentration is 100–250 µM and siRNA should be dissolved in RNase-free water.
The volume of siRNA solution should be less than 10% of cell solution to avoid drastic changes in ion concentrations or osmotic pressure.
For siRNA transfection, required siRNA concentration may vary depending on the efficacy of the siRNA duplex
The siRNA concentration may need to be optimized depending on the target gene of the user.

Electroporation system

Materials

In this section, general guidelines for operating the ExTransfection™ to transfect mammalian cells are described. "Optimization Protocols" are described in the next chapter.

Please prepare the following materials before starting electroporation.

- ExTransfection™ kits
- Cell solutions at **$1x10^7$** cells/mL for adherent cell types and at **$2x10^7$** cells/mL for suspension cell types

⚠ ***CAUTION***

Please make sure to use correct resuspension buffer depending on the voltage to be used. For a protocol using voltage less than 1900V, use resuspension buffer R, and for a protocol using voltage 1900V or higher, use resuspension buffer T.

- High quality DNA at a concentration of 1–5 µg/µL (in deionized water or TE buffer), or high quality RNAi duplex at a concentration of 100–250 µM (in nuclease-free water)
- Cell culture plates filled with media without antibiotics

Not Supplied

1. Cells for the experiment
2. High-purity transfection payload (e.g., DNA, RNA)
3. Cell culture plates containing appropriate growth medium
4. D-PBS or PBS without calcium (Ca^{2+}) and magnesium (Mg^{2+})
5. Trypsin/EDTA or equivalent reagent for adherent cell detachment
6. Automated cell counter for cell lines and primary cells
7. Instruments and assays required for result analysis and cell characterization

Recommended amounts for electroporation

Recommended amounts of plasmid DNA or siRNA for cell types and vessel types are shown in **Table** 1 (Page 46).

Electroporation system

Materials

Fill ExTransfection™ tube with buffer
Fill ExTransfection™ tube with 3 mL of buffer E for 10 µL ExTransfection™ Tip, and 3 mL of buffer E2 for 100 µL ExTransfection™ Tip.

Set Pulse Conditions
Set pulse conditions on ExTransfection™.

Aliquot DNA/siRNA and Add cell solution
Add the calculated DNA/siRNA to the tube containing the cell solution and mix gently. Recommended amounts for cell concentration, DNA, and media volume can be found in **Table 1** (Page 46).

⚠ ***CAUTION***

- It is highly recommended to optimize cell concentrations, DNA/siRNA concentrations, and electroporation parameters to achieve the best results. Refer to page **47** for Optimization Protocol.
- Ensure that the amount of DNA/RNA in the
- Transfection mixture does not exceed 10% of the total volume.
- It is important to use actively dividing cells to achieve best results. Often times, for adherent cells, using a flask of cells with 70~80% confluence is recommended.

※ It is important to avoid bubbles within tips which may cause arcs during electroporation. Therefore, it is important to prepare enough volume of samples so that tip can be completely submerged through the last pipetting.

※ When loading samples into a tip, make sure that the sample solution has been well mixed.

Electroporation system

Table 1. Recommended amounts of cells, DNA or siRNA, and media for vessel types.

Format	Cell Type	DNA (µg)	siRNA (nM)	ExTransfection™ Tip	Vol. plating medium	Cell no. / well	Buffer R or Buffer T[1]
96-well	Adherent	0.25–0.5	10–200	10 µL	100 µL	1–2 × 10^4	10 µL/well
	Suspension	0.5–1		10 µL		2–5 × 10^4	10 µL/well
48-well	Adherent	0.25–1	10–200	10 µL	250 µL	2.5–5 × 10^4	10 µL/well
	Suspension	0.5–2		10 µL		5–12.5 × 10^4	10 µL/well
24-well	Adherent	0.5–2	10–200	10 µL	500 µL	0.5–1 × 10^5	10 µL/well
	Suspension	0.5–3		10 µL		1–2.5 × 10^5	10 µL/well
12-well	Adherent	0.5–3	10–200	10 µL	1 mL	1–2 × 10^5	10 µL/well
	Suspension	0.5–3		10 µL		2–5 × 10^5	10 µL/well
6-well	Adherent	0.5–3 (10 µL) 5–30 (100 µL)	10–200	10 µL/100 µL	2 mL	2–4 × 10^5	10 µL or 100 µL/well
	Suspension	0.5–3 (10 µL) 5–30 (100 µL)		10 µL/100 µL		0.4–1 × 10^6	10 µL or 100 µL/well
60 mm	Adherent	5–30	10–200	100 µL	5 mL	0.5–1 × 10^6	100 µL/well
	Suspension	5–30		100 µL		1–2.5 × 10^6	100 µL/well
10 cm	Adherent	5–30	10–200	100 µL	10 mL	1–2 × 10^6	100 µL/well
	Suspension	5–30		100 µL		2–5 × 10^6	100 µL/well

Optimization

Description

DNA & siRNA Optimization Protocols

Electroporation mainly relies on a combination of three electrical parameters: electric field, pulse duration, and number of pulses. It may be necessary to optimize these electroporation parameters for each cell type, especially when a certain cell type is difficult to transfect.

ExTransfection™ offers a pre-programmed optimization protocol that helps an easy and quick optimization of electrical parameters for both adherent and suspension cells.

For PBMCs, refer to the protocol described in page 51.

General guideline for optimization

1. Run initial 24-well based optimization using pre-programmed ranges of electrical parameters.

2. Based on the initial optimization results, perform a second optimization or repeat the 24-well based optimization for verification.

3. If needed, further adjust the range of electrical parameters based on the second optimization results to proceed with option; day three, or modify the concentration of DNA or siRNA, and repeat the 24-well based optimization to determine the optimal working conditions.

Optimization

DAY-1 (Adherent & Suspension Cell Lines)

24-well Optimization protocol DAY-1 (Adherent and Suspension Cell Lines)

1. After preparing cells, and DNA or siRNA as described in pages 39-43, prepare a 24-well plate filled with 0.5mL of cell culture media containing serum but no antibiotics. Prepare enough cells and plasmid DNA/siRNA for at least 30 electroporations.

2. Refer to the table below for the number of cells and the amount of DNA or siRNA for running optimization using 10 µL ExTransfection™ tips. If 100 µL ExTransfection™ tips are used, prepare up to 10 times more.

Table 2. Recommended preparation amount by cell type.

Cell type	Cell no.	DNA (1µg/µl)	siRNA	Resuspension Buffer R
Adherent	1×10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	10 µL/well 285 µL/plate
Suspension	2×10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	10 µL/well 285 µL/plate

***TIP**: Highly recommended to prepare at least 10% extra amount of substances (cells, DNA or siRNA, and buffer) in case of sample loss.*

Optimization

DAY-1 (Adherent & Suspension Cell Lines)

Table 3. 24-well optimization parameters and record table (R buffer)

Sample	Well no.	Pulse voltage	Pulse width	Pulse no.	Results	
					Transfection efficiency	Cell viability
1	A1	Use pre-optimized parameter or control without electroporation.				
2	A2	1400	20	1		
3	A3	1500	20	1		
4	A4	1600	20	1		
5	A5	1700	20	1		
6	A6	1100	30	1		
7	B1	1200	30	1		
8	B2	1300	30	1		
9	B3	1400	30	1		
10	B4	1000	40	1		
11	B5	1100	40	1		
12	B6	1200	40	1		
13	C1	1100	20	2		
14	C2	1200	20	2		
15	C3	1300	20	2		
16	C4	1400	20	2		
17	C5	850	30	2		
18	C6	950	30	2		
19	D1	1050	30	2		
20	D2	1150	30	2		
21	D3	1300	10	3		
22	D4	1400	10	3		
23	D5	1500	10	3		
24	D6	1600	10	3		

3. In an ExTransfection™ tube, fill 3 mL of Buffer E for 10µL tips and Buffer E2 for 100 µL tips, respectively. Assemble ExTransfection™ tube into ExTransfection™ pipette station.

4. Aspirate cell-DNA/siRNA mixture into a tip and place ExTransfection™ pipette into the ExTransfection™ tube.

5. Press the "Optimization" button to load optimization protocol and using the parameters listed above, run electroporation.

Optimization

DAY-1 (Adherent & Suspension Cell Lines)

6. After electroporation is complete, transfer the cell solution into the wells of the 24-well plate in order.

NOTE: When using the 100 µL ExTransfection™ tip, dilute the sample 10-fold by adding it to 900 µL of medium, then transfer 100 µL of the sample to the pre-warmed 0.4 mL culture medium.

7. Repeat steps 4 to 6 to test the range of electroporation parameters (voltage, width, and pulse) and fill the 24-well plate.

8. Gently shake the plate to ensure that cells are evenly distributed. Keep the plate in a CO_2 incubator for at least 24 hr.

Measure transfection efficiency using fluorescence microscopy or functional analysis. Select a set of parameters with best efficiency and proceed to Optimization Day-2 protocol.

Optimization

DAY-1 (Primary suspension blood cells)

18-well Optimization protocol DAY-1 (primary suspension blood cells)

1. After preparing cells, and DNA or siRNA as described in pages 39-43, prepare a 24-well plate filled with 0.5 mL of cell culture media containing serum but no antibiotics. Prepare enough cells and plasmid DNA/siRNA for at least 20 electroporations.

2. Refer to the table below for the number of cells and the amount of DNA or siRNA for running optimization using 10 µL ExTransfection™ tips.

Table 4. Recommended preparation amount by cell type.

Cell type	Cell no.	DNA (1µg/µl)	siRNA	Resuspension Buffer T
Primary blood suspension cells	2 × 10^5/well	0.5–1 µg DNA/well 20 µg/plate	50-100 pmol in 10 µL tip 100-200 nM per well	**Buffer R/T** 10 µL/well 180 µL/plate

3. In an ExTransfection™ tube, fill 3 mL of Buffer E. Assemble ExTransfection™ tube into ExTransfection™ pipette station.

4. Aspirate cell-DNA/siRNA mixture into a tip and place ExTransfection™ pipette into the ExTransfection™ tube.

Optimization

DAY-1 (Primary suspension blood cells)

Table 5. 18-well optimization parameters and record table (T buffer)

Sample	Well no.	Pulse voltage	Pulse width	Pulse no.	Results	
					Transfection efficiency	Cell viability
1	A1	Use pre-optimized parameter or control without electroporation.				
2	A2	2000	20	1		
3	A3	2050	20	1		
4	A4	2100	20	1		
5	A5	2150	20	1		
6	A6	2200	20	1		
7	B1	2250	20	1		
8	B2	2300	20	1		
9	B3	2350	20	1		
10	B4	2400	15	1		
11	B5	2450	15	1		
12	B6	2500	15	1		
13	C1	2000	15	2		
14	C2	2050	15	2		
15	C3	2100	15	2		
16	C4	2150	15	2		
17	C5	2200	15	2		
18	C6	2250	15	2		

5. Enter the electroporation parameters in the Input window and perform electroporation using the parameters listed above.

6. After electroporation is complete, transfer the cell solution into the wells of the 24-well plate in order.

7. Repeat steps 4 to 6 with the remaining sample to test the range of electroporation parameters (voltage, width, and pulse) and fill the 18-well.

8. Gently shake the plate to ensure that cells are evenly distributed. Keep the plate in a CO_2 incubator for at least 24 hr.

Measure transfection efficiency using fluorescence microscopy or functional analysis. Select a set of parameters with best efficiency and proceed to Optimization Day-2 protocol.

Optimization

DAY-2

Optimization protocol DAY-2

Select the best transfection parameters from Optimization Protocol DAY-1. In DAY-2, set ranges of electroporation parameters (voltage, width and pulse) which are narrower than DAY-1. Test new lists of parameters. For details, refer to the Optimization Protocol DAY-1. Refer to the table below for the number of cells and the amount of DNA or siRNA for running optimization using 10 µL ExTransfection™ tips. When using 100 µL ExTransfection™ tips, prepare 10 times more volume or amount.

Table 6. Recommended preparation amount by cell type.

Cell type	Format	Cell no.	DNA(1µg/µl)	siRNA	Resuspension Buffer
Adherent	24-well	1 × 10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	**Buffer R** 10 µL/well 285 µL/plate
Suspension	24-well	2 × 10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	**Buffer R** 10 µL/well 285 µL/plate
Primary blood suspension cells	18-well	2 × 10^5/well	0.5–1 µg DNA/well 20 µg/plate	50-100 pmol in 10 µL tip 100-200 nM per well	**Buffer R/T** 10 µL/well 180 µL/plate

Optimization

DAY-2

Please refer to the example of the refinement method for DAY-2 in the table below and proceed accordingly.

• Example: When the optimal parameter selected from the DAY-1 protocol falls between 1,500 V, 20 ms and 1,400 V, 30 ms.

Table 7. Example of Day 2 optimization parameters and documentation table

Sample	Well no.	Pulse voltage	Pulse width	Pulse no.	Results	
					Transfection efficiency	Cell viability
1	A1	1450	10	2		
2	A2	1475	10	2		
3	A3	1500	10	2		
4	A4	1525	10	2		
5	A5	1550	10	2		
6	A6	1575	10	2		
7	B1	1375	10	3		
8	B2	1400	10	3		
9	B3	1425	10	3		
10	B4	1450	10	3		
11	B5	1475	10	3		
12	B6	1500	10	3		
13	C1	Control containing DNA but no electroporation pulse.				

Perform electroporation by referring to the example parameters listed in the table.

If needed, repeat refinement optimization protocol until finding the best electroporation parameters.

TIP: When the best electroporation parameters have been identified, save the parameters.

Maintenance and Cleaning

Maintenance and Cleaning

Wipe the surface of ExTransfection™ and ExTransfection™ pipette station with a damp cloth. Do not use detergents or organic solvents to clean. ExTransfection™ pipettes has been calibrated during manufacturing processes and does not require additional calibration.

Be careful not to let liquid sip into ExTransfection™ pipette station. Liquid around ball plunger may cause rust building up around the plunger.

If liquids (e.g. buffer, water, coffee) accidentally spill into ExTransfection™ pipette station, remove pipette station from the main unit and wipe liquids out, and turn pipette station upside down to dry completely for 24 hours. Do not use oven to dry ExTransfection™ pipette station. If ExTransfection™ pipette station does not work even after drying, contact technical support.

If one needs any other repairs or service, please contact technical support. Do not attempt to repair or service ExTransfection™. Doing so may cause damage to ExTransfection™.

⚠ **IMPORTANT! Never disassemble or service ExTransfection™ by oneself.**

Unauthorized repairs may damage ExTransfection™ or alter its functionality, which will void one's warranty. Contact **sales@nanoentek.com** or one's local distributor to arrange for service.

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

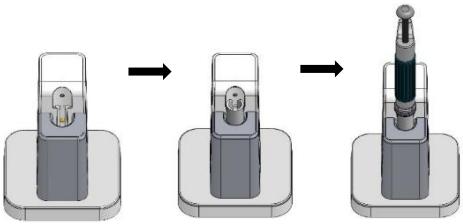
Do not directly spray ethanol anywhere on ExTransfection™.

⚠ **IMPORTANT! Avoid exposing ExTransfection™ to UV light.**

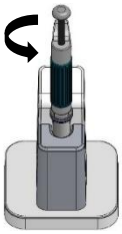
UV light may degrade components, including plastic. Damage from UV exposure is not covered under the manufacturer's warranty

Replace the pipette gripper

1. Put an ExTransfection™ tube into ExTransfection™ pipette station, and put ExTransfection™ pipette into ExTransfection™ pipette station all the way down until there is a “click” sound.



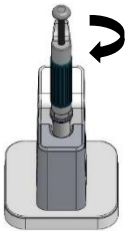
2. Turn ExTransfection™ pipette gripper in counterclockwise and remove ExTransfection™ pipette gripper. Turn ExTransfection™ pipette gripper in counterclockwise and remove ExTransfection™ pipette gripper.



3. Assemble a new gripper and springs.

Replace the pipette gripper

4. Put a new gripper back to ExTransfection™ pipette, and turn the gripper in clockwise.



5. As shown in the figure below, make sure to align the finger rest part of the pipette ① and the connector to ball plunger ②.

Troubleshooting

ExTransfection™ Error messages

The table below describes error messages that may pop up on ExTransfection™ screen. One will be able to continue to use ExTransfection™ after fixing error(s). If ExTransfection™ needs to be repaired, please contact sales@nanoentek.com or one's local distributor.

Error message	Action
Please connect station	Check all the connections between ExTransfection™ pipette station and ExTransfection™ unit.
Check tip for air bubbles.	Remove air bubbles trapped in the tip by discarding cell solution in the tip and aspirating once again slowly to avoid air bubbles.
Please enter protocol name	When storing a protocol in a database, one needs to enter a protocol name.
Password incorrect, please re-enter	Re-enter the 4-digit password and press OK to exit the screen.
Input voltage, pulse width, or pulse number error	One or more of electroporation parameters are out of range.

No power (the display remains blank even after the device is turned on)	
Check the DC power Jack connection	Check the DC power Jack connections on both ends. Be sure to use the correct power cord.

Connection errors	
Pipette or tube is incorrectly inserted	Check if ExTransfection™ tube is properly inserted into ExTransfection™ pipette station. (Refer to page 27) Check if ExTransfection™ pipette is properly inserted into ExTransfection™ pipette station. (Refer to page 29) Check if there is any spill or debris around electrodes or ball plunger on ExTransfection™ pipette or ExTransfection™ pipette station.
The sensor connector is not connected	Check if the sensor connector cable from ExTransfection™ pipette station is properly connected to the sensor port in the back of ExTransfection™ unit. (Refer to page 24)
High voltage connector is not connected	Check if the high voltage connector cable from ExTransfection™ pipette station is properly connected to the high voltage port in the back of ExTransfection™ unit. (Refer to page 24)

Troubleshooting

Connection Failure	
No ExTransfection™ tip is inserted or the ExTransfection™ tip is inserted incorrectly	Check if ExTransfection™ tip is properly inserted into ExTransfection™ pipette. (Refer to page 28)
No buffer in the tube or no sample in the tip	Make sure to add 3mL of electrolytic buffer to ExTransfection™ tube. Make sure to prepare cell solution in Resuspension buffer. Make sure to aspirate cell solution mixed with DNA or siRNA into ExTransfection™ tip.
Wrong buffers used	Make sure to use Buffer E for 10 µL tips and Buffer E2 for 100 µL tips. Make sure to use only the buffers included in ExTransfection™ kits.
If the error persists even when all connections are correct	
Run self-diagnostics	Click ✓ the Self-diagnosis button in the Setting Tab to run the self-diagnostic test. During the self-diagnosis process, the device performs various checks to identify any issues. If the self-test result shows "OK" but the device still does not operate properly, please contact your service representative or email sales@nanoentek.com.

Sparks	
Air bubbles trapped in ExTransfection™ tip	When air bubbles are trapped in ExTransfection™ tip, there can be sparks. Avoid bubbles in tips.
Too high voltage or too many pulses	When electroporation is performed at too high voltage or for too long, there can be sparks. Reduce voltage or pulse setting
Accidentally used salt-precipitated DNA	If DNA is prepared using salt-precipitation, sparks may occur due to salt contamination. When concentrating DNA, be sure not to use the ethanol-precipitation method.

Troubleshooting

Low cell survival rate	
Poor DNA quality	Use high-quality plasmid DNA for transfection (Refer to page 42 for guidelines and recommendations on DNA quality)
Cells are stressed or damaged	When harvesting cells, avoid excessive stresses such as high-speed centrifugation, or vigorous pipetting. Avoid using cells that has been cultured to be over-confluent. After electroporation, immediately incubate cells in warm, antibiotic-free media
Repetitive uses of same ExTransfection™ tip	When ExTransfection™ tip is used for more than twice, electrodes in the tip may be degraded and transfection efficiency may decrease.

Low transfection efficiency	
Poor optimization of electroporation parameters	Please refer to the optimization protocol to find the best conditions. Following the detailed procedures can help you identify the optimal settings.
Poor quantity or quality of DNA or siRNA	Use right amount of high-quality DNA for better transfection results (Refer to page 42 for guidelines and recommendations on DNA quality)
Incorrect cell density	When cell concentrations are either too low or too high, transformation efficiency may decrease.
Contaminated cells	When cells are contaminated either by bacteria or mycoplasma, transfection efficiency may decrease.

High variabilities in transfection efficiency	
Cell status such as passage number or confluency	It is highly recommended to use cells with a low passage number and maintain confluency below 80%. To achieve consistent results, using cells under the same conditions can be advantageous.
Multiple uses of ExTransfection™ tube	Do not use a same ExTransfection™ tube more than 10 times. If using different plasmid DNA or cell samples, always use a new ExTransfection™ tip and tube to avoid cross-contamination.

High energy error	
Used too high electrical parameters	Lower voltage, width, and pulse.

Warranty

To ensure the safe operation of the ExTransfection™ Electroporation System, follow the guidelines provided in this section. This device is designed in compliance with EN61010-1 Safety Standards. For reliable and secure use, always adhere to the instructions outlined in this manual. Failure to do so may result in safety hazards and will invalidate both the manufacturer's warranty and the EN61010-1 safety certification. NanoEntek assumes no responsibility for any injuries or damages caused by improper use of this instrument. Repairs and maintenance should only be conducted by authorized NanoEntek personnel.
If any defects occur in ExTransfection™, 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 any inquiry or request for repair service, please contact **sales@nanoentek.com** or one's local distributor.

Documentation

- User guides, manuals, and protocols
- Certificates of Analysis
- Safety Data Sheets (SDSs; also known as MSDSs)

Safety

Safety information

Review and follow the safety instructions below:

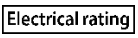
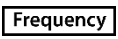
- If water or other material enters the instrument, the adaptor, or power inlet, disconnect the power cord and contact a service person. For operating environment, refer to Product Specifications.
- Do not touch the main plug or power cord with wet hands.
- Always ensure that the input voltage matches the local power supply
- 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 does 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 DC 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.

Safety Symbols

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

Symbol	Meaning
	Caution & Warning
	Protective earth (Ground)
	This equipment has been tested and found to comply with the limits for a Class A digital instrument, 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 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.
	CE marking
	Risk of electrical shock
	The regulatory compliance mark indicates that it complies with Australian standards for electromagnetic compatibility
	Consult Instructions for Use An electronic instructions for us (eIFU) indicator (website address) may accompany the symbol when used to indicate an instruction to consult an eIFU.
	Storage temperature
	Lot number/Batch number
	Electrical rating
	Use by YYYY-MM-DD or YYYY-MM
	Frequency is the rate at which current changes direction per second.

REF	Catalogue number/Reference number
SN	Serial number
	Manufacturer
	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.
	Keep dry Keep away from rain
	Fragile, handle with care
	This way up
	General symbol for recover/recyclable
	Team lift
European Corporation	European Corporation
US Corporation	US Corporation

Safety sheet

Chemical safety

⚠ **Warning! Precautions for handling chemical substances**

To minimize risk, one should ensure that one's laboratory personnel are familiar with and follow the basic safety guidelines for the use, storage, and disposal of chemicals below. For detailed guidance on these chemicals, please refer to the relevant Safety Data Sheet (SDS):

Before working with chemicals, read and understand the Safety Data Sheets (SDS) provided by the manufacturer. SDS can be found in the "Documentation & Support" section of this document.

Minimize direct contact with chemicals and be sure to wear appropriate protective equipment (e.g., safety glasses, gloves, protective clothing) when handling them.

Do not inhale vapors or mist of chemicals. Keep chemical containers sealed at all times and, if necessary, work in a well-ventilated area (e.g. hood).

Periodically check for chemical leaks or spills. If an accident occurs, one must follow the accident remediation procedures as suggested by the SDS.

Dispose of chemical waste safely in the hood.

Use separate waste containers into primary and secondary containers. The primary container is the container for direct waste, and the secondary container is the container for any leaks or spills that occur in the primary container. Both containers are compatible with waste materials and must comply with all relevant legal requirements.

After emptying the waste container, be sure to seal it with the lid provided and dispose of it.

Properly analyze the waste generated by the chemicals, reagents, and substrates used to determine their properties.

Save, transport, and dispose of all waste in accordance with the relevant regulations of one's local, state/province and country.

⚠ **Attention!** Radioactive or biohazard materials require special handling and may be restricted when disposed of.

⚠ **Warning! Hazardous waste (waste from the appliance)**

Waste generated by the appliance can be hazardous. Be sure to follow the instructions provided in the warning for "Handling General Chemicals".

⚠ **Warning! 4 liter reagent and waste container safety**

Reagents and waste in a 4 liter container may crack or leak. Each 4-litre container must be fastened in a low-density polyethylene safety case, with the lid locked and the handle fixed upwards.

Safety sheet

Biological hazard safety

⚠ **Warning! Potential biohazard**

Depending on the sample used, the surface of this instrument may constitute a biological hazard. When handling biohazard materials, proper decontamination procedures must be followed.

⚠ **Warning! Biohazard**

Biological samples such as tissues, bodily fluids, infectious substances, and human and animal blood are at risk of transmitting infectious diseases. All work must be carried out in a facility equipped with appropriate safety equipment (e.g., physical barriers). Safety equipment may include gloves, coveralls, gowns, shoe covers, boots, respiratory protection, face shields, safety glasses or goggles, etc. Before dealing with biohazard materials, it is important to be trained in accordance with the relevant regulations and the requirements of the agency. Workers must comply with national, state, and local regulations. The main data to refer to when handling biological samples are:

- U.S. Department of Health and Human Services, Biosafety Manual for Microbiology and Biomedical Laboratories, 6th Edition, HHS Issue Number (CDC) 300859, revised June 2020 CDC Biosafety Manual

  https://www.cdc.gov/labs/bmbl/index.html

- Laboratory Biosafety Manual, 4th Edition, World Health Organization (WHO), 2020 WHO Biosafety Manual

  www.who.int/publications/i/item/9789240011311

Product specifications

ExTransfection™ Electroporation System, along with the ExTransfection™ pipette station, is designed to work with typical nonhazardous laboratory reagents. Avoid using organic solvents in the tips, tubes, or with the device.

Items	Specification
Operating Power	24VDC, 7.5A, (Adaptor)
Output	0.5-2.5 kV
Pulse Width	1-100 ms
Maximum Duty Cycle	0.1
Charging Time	Maximum 8 seconds
Operating Temperature	5℃ to 40℃
Maximum Relative Humidity	Up to 80%
Degree of Protection	IPX0
Means of Protection	Class III
Installation Category	II
Instrument Type	Benchtop unit
Device Dimensions	251.6 (W) x 383.4 (D) x 185.1 (H) mm 9.9 (W) x 15.1 (D) x 7.3 (H) inches
Pipette Station Dimensions	108.5 (W) x 104.6 (D) x 130 (H) mm 4.27 (W) x 4.12 (D) x 5.12 (H) inches
Weight (Device & Pipette Station)	15.4 pounds (7kg)
Display	8 inches Capacitive Touch (1920 x 1080 pixels)

Ordering information

The following products are essential used with ExTransfection™ Electroporation System. For more information, visit NanoEntek.com or contact Technical Support.

Cat. No.	Product	Description
EXT1000	ExTransfection, Electroporation System	Main device, Pipette, Pipette station
EXT1000P	ExTransfection Pipette	Pipette (1 ea)
EXT1000PS	ExTransfection Pipette Station	Pipette Station (1 ea)
EXT1025K	ExTransfection 10 µL Kit	Resuspension buffer R (1 ml)
		Resuspension buffer T (1 ml)
		Electrolytic buffer E (75 ml)
		Disposable tip (10 µL, 25 tips)
		Disposable tube (5 ea)
EXT10025K	ExTransfection 100 µL Kit	Resuspension buffer R (10 ml)
		Resuspension buffer T (10 ml)
		Electrolytic buffer E2 (75 ml)
		Disposable tip (100 µL, 25 tips)
		Disposable tube (5 ea)
EXT1096K	ExTransfection 10 µL Kit	Resuspension buffer R (1 ml, 3 ea)
		Resuspension buffer T (1 ml, 3 ea)
		Electrolytic buffer E (150 ml, 2 ea)
		Disposable tip (10 µL, 96 tips)
		Disposable tube (20 ea)
EXT10096K	ExTransfection 100 µL Kit	Resuspension buffer R (30 ml)
		Resuspension buffer T (30 ml)
		Electrolytic buffer E2 (150 ml × 2 ea)
		Disposable tip (100 µL, 96 tips)
		Disposable tube (20 ea)
EXT50T	ExTransfection Tube	Disposable tube (5 ea, 10 packs)

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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ExTransfection™

Electroporation system

NESMU-EXT-001EN (V.0.0)

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