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Neon™ NxT Electroporation System

USER GUIDE

For electroporation of mammalian cells, including primary and stem cells, with high transfection efficiency

Catalog Numbers NEON18S, NEON1S, NEON18SK, NEON1SK

Publication Number MAN0026677

Revision B.0

ThermoFisher SCIENTIFIC

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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

Revision history: B.0 MAN0026677 (English)

Revision	Date	Description
B.0	22 October 2024	Addition of new Resuspension Genome Editing Buffer, and 8-channel pipette and pipette station.
A.0	22 March 2023	New product manual for the Neon™ NxT Electroporation System.

The information in this guide is subject to change without notice.

Contents

1 Product information

Product description

The Neon™ NxT Electroporation System is a benchtop electroporation device that employs an electroporation technology which uses a pipette tip as an electroporation chamber to efficiently transfect mammalian cells including primary and immortalized hematopoietic cells, stem cells, and primary cells.

The Neon™ NxT Electroporation System efficiently delivers nucleic acids, proteins, and siRNA into all mammalian cell types with a high cell survival rate. The transfection is performed using as few as 1×10^4 or as many as 1×10^7 cells per reaction in a volume of 10 µL or 100 µL for a variety of cell culture formats (60 mm, 6-well, 12-well, 24-well, and 96-well).

The Neon™ NxT Electroporation System uses a single transfection kit (Neon™ NxT Kit) that is compatible with various mammalian cell types including primary and stem cells thereby avoiding the need to determine an optimal buffer for each cell type.

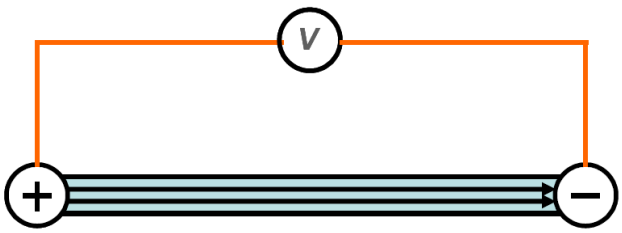
The Neon™ NxT Electroporation System uses open and transparent protocols that are optimized for ease of use and simplicity. The Neon™ NxT device is preprogrammed with 24 optimization protocols, and over 400 preprogrammed cell-specific protocols. In addition, there is capacity for 10,000 more protocols in the Neon™ NxT device database. Optimized protocols for many commonly used cell types are also available at **thermofisher.com/transfectionprotocolsandcitations** to maximize transfection efficiencies for your cell types.

See “System components” on page 11 for details on various parts of the system.

System overview

Unlike standard cuvette based electroporation, the Neon™ NxT Electroporation System uses a unique electroporation reaction chamber, the Neon™ NxT Tip that delivers a high electric field to the biological sample. The Neon™ NxT Tip maximizes the gap size between the two electrodes while minimizing the surface area of each electrode. As a result, the sample experiences a more uniform electric field, minimal pH change, less ion formation, and negligible heat generation.

The established Neon™ electroporation technology overcomes limitations associated with standard cuvette based electroporation to increase transfection efficiency and cell viability, and provide an ergonomic workflow.



Features

Important features of the Neon™ NxT Electroporation System are listed below:

- Small footprint—the benchtop design fits inside a tissue culture hood, reducing contamination risk.
- Preserves samples—minimize sample transfer loss with the complete elimination of an electroporation cuvette and associated pipetting steps.
- Flexible—deliver DNA, RNA, and protein to 1×10^4 to 1×10^7 cells per reaction in a sample volume of 10 µL or 100 µL.
- Customizable—optimize electroporation parameters freely, or leverage over 400 preprogrammed protocols with the ability to save up to 10,000 more protocols.
- Simplicity—easy to perform workflow with only three steps; a single reagent kit is all that is needed for most cell types and delivery payloads.
- Connectivity—the TransfectionLab app paired with the Thermo Fisher™ Connect Platform (**apps.thermofisher.com/apps/spa/#/dashboard**) allows for digital experiment design and connectivity to the Neon™ NxT device.
- Improved usability—pipette ergonomics with ClipTip™ technology for loading and unloading of the pipette tips, enhanced feedback loop, and intuitive user interface with plate setup.

Product contents

Neon™ NxT System contents

The contents of the Neon™ NxT Electroporation System are listed in the following table. The Neon™ NxT Electroporation System is shipped at room temperature.

See page 11 for descriptions of the Neon™ NxT Electroporation System, and page 37 to set up the device.

Neon™ NxT Electroporation System with 1-Channel Pipette (Cat. No. NEON1S, NEON1SK [1])	Quantity
Neon™ NxT Electroporation Device	1
Power Cord (for US/Canada/Taiwan/Japan, Europe, or UK)	1
Neon™ NxT 1-Channel Pipette	1
Neon™ NxT 1-Channel Pipette Station	1
Quick Reference	1

[1] Starter kits include two Neon™ NxT Kits (Cat. Nos. N1096, N10096).

Neon™ NxT Electroporation System with 1-Channel and 8-Channel Pipettes (Cat. No. NEON18S, NEON18SK [1])	Quantity
Neon™ NxT Electroporation Device (8-Channel Software Activation pre-installed)	1
Power Cord (for US/Canada/Taiwan/Japan, Europe, or UK)	1
Neon™ NxT 1-Channel Pipette	1
Neon™ NxT 1-Channel Pipette Station	1
Neon™ NxT 8-Channel Pipette	1
Neon™ NxT 8-Channel Pipette Station	1
Quick Reference	1

[1] Starter kits include two Neon™ NxT Kits (Cat. Nos. N1096-8, N10096).

Neon™ NxT Electroporation System 8-Channel Upgrade Package (Cat. No. NEON8/NEON8U)	Quantity
Neon™ NxT 8-Channel Pipette	1
Neon™ NxT 8-Channel Pipette Station	1
Quick Reference, Neon NxT 8-Channel Software Activation Instructions	1

Neon™ NxT Kit contents

Neon™ NxT Kits are used with the Neon™ NxT Electroporation System for efficient transfection of mammalian cells and are available as standalone products (see “Accessory products” on page 85). The kits consist of two components which are not sold individually (a Tips/Tubes Kit, and a Buffer Kit), and are available in two formats (for electroporation of 10 µL samples, and 100 µL samples).

Neon™ NxT Kit components are listed in the following table, and are shipped at room temperature.

Store buffers at 4°C and protected from light to maintain their stability and functionality..

Note: Temperature fluctuations can lead to buffer precipitation. Precipitate does not dissolve by incubation at 37°C, so if observed, removal of the precipitate by filtration before proceeding with the electroporation process is recommended. It is important to note that precipitation does not affect the functional properties of the buffers.
Do not make aliquots of buffers in other containers for long-term storage, as this may compromise stability and performance.

Store tips/tubes at room temperature.

Catalog numbers that appear as links open the web pages for those products.

Table 1 Consumables for use with the Neon™ NxT 1-Channel Pipette

Item	Neon™ NxT Kit, 10 µL		Neon™ NxT Kit, 100 µL	
	Cat. No. N1025 (50 reactions)	Cat. No. N1096 (192 reactions)	Cat. No. N10025 (50 reactions)	Cat. No. N10096 (192 reactions)
Tips/Tubes Kit	N1025K	N1096K	N10025K	N10096K
Neon™ NxT Tips	25 tips (10 µL)	96 tips (10 µL)	25 tips (100 µL)	96 tips (100 µL)
Neon™ NxT 1-Channel Tubes	8	32	8	32
Buffer Kit	N1025B	N1096B	N10025B	N10096B
Neon™ NxT Resuspension R Buffer (Proprietary)	1 mL	4 × 1 mL	10 mL	4 × 10 mL
Neon™ NxT Resuspension T Buffer (Proprietary)	1 mL	4 × 1 mL	10 mL	4 × 10 mL
Neon™ NxT Resuspension Genome Editing Buffer (Proprietary)	1 mL	4 × 1 mL	10 mL	4 × 10 mL
Neon™ NxT Electrolytic E10 Buffer (Proprietary)	50 mL	2 × 100 mL	—	—
Neon™ NxT Electrolytic E100 Buffer (Proprietary)	—	—	50 mL	2 × 100 mL

Table 2 Consumables for use with the Neon™ NxT 8-Channel Pipette

Item	Neon™ NxT Kit, 10 µL		Neon™ NxT Kit, 100 µL	
	Cat. No. N1096-8 (192 reactions)	Cat. No. N10384-8 (768 reactions)	Cat. No. N10096-8 (192 reactions)	Cat. No. N100384-8 (768 reactions)
Tips/Tubes Kit	N1096K8	N10384K8	N10096K8	N100384K8
Neon™ NxT Tips	96 tips (10 µL)	384 tips (10 µL)	96 tips (100 µL)	384 tips (100 µL)
Neon™ NxT 8-Channel Tubes	4	16	4	16
Buffer Kit	N1096B	N10384B	N10096B	N100384B
Neon™ NxT Resuspension R Buffer (Proprietary)	4 × 1 mL	2 × 10 mL	4 × 10 mL	2 × 80 mL
Neon™ NxT Resuspension T Buffer (Proprietary)	4 × 1 mL	2 × 10 mL	4 × 10 mL	2 × 80 mL
Neon™ NxT Resuspension Genome Editing Buffer (Proprietary)	4 × 1 mL	2 × 10 mL	4 × 10 mL	2 × 80 mL
Neon™ NxT Electrolytic E10 Buffer (Proprietary)	2 × 100 mL	4 × 200 mL	—	—
Neon™ NxT Electrolytic E100 Buffer (Proprietary)	—	—	2 × 100 mL	4 × 200 mL

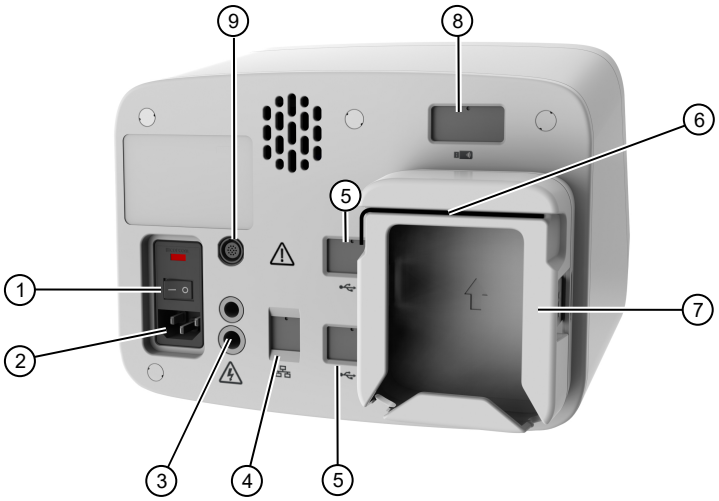
System components

Neon™ NxT device

The electrical parameters of the Neon™ NxT device are factory set and do not require calibration. It is used with Neon™ NxT Pipette Stations and Neon™ NxT Kits to efficiently transfect mammalian cells including primary and stem cells.

Front and rear view

① Status indicator LED
② Touchscreen



① Power switch
② AC inlet (connect to the power cord, and plug into a power outlet)
③ High voltage port (connect to the high voltage connector on one end of the power cord)
④ Ethernet port (covered port; use a hex key for access)
⑤ USB 2.0 port (covered port; use a hex key for access)
⑥ Hex key (Allen wrench)
⑦ Connector cable storage
⑧ USB wireless adaptor port
⑨ Low voltage interface port (connects to the low voltage interface connector on one end of the power cord)

LED status indicators

LED color	Status
Blue	Instrument is idle.
Green	Instrument is in operation.
Blinking green	Instrument high voltage subsystem in operation.
Blinking amber	Warning/error

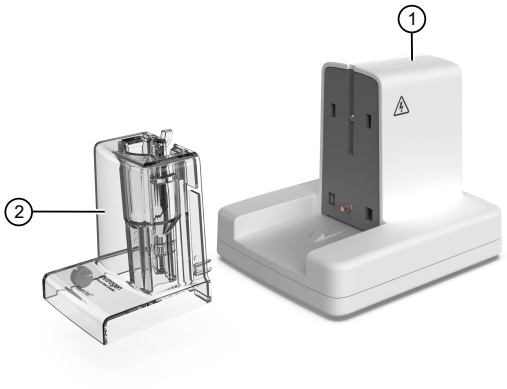
About the covered ports

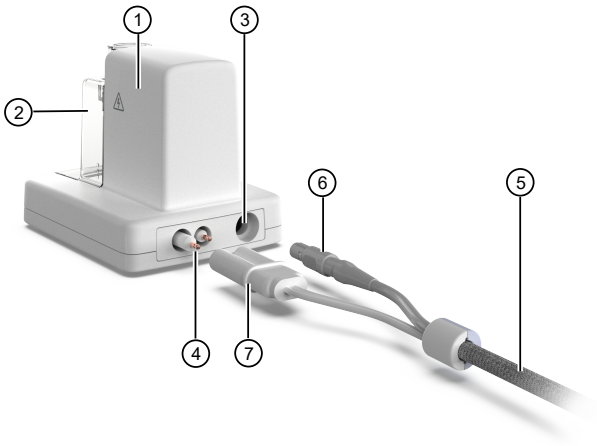
Do not access the covered ports at the rear of the device during electroporation. The ports should remain covered during the protocol, and should only be accessed when the device is in idle mode.

To open the covers over the ports, place one end of the supplied wrench into the hole located in the cover and slide it down to expose the port.

Neon™ NxT 1-Channel Pipette Station

The Neon™ NxT 1-Channel Pipette Station holds a Neon™ NxT 1-Channel Pipette during electroporation procedures. The pipette (with attached tip) is held in the Neon™ NxT 1-Channel Tubes which has an electrode near the bottom that transfers the electric field from the electrode inside the Neon™ NxT Tip.





① Neon™ NxT 1-Channel Pipette Station
② Tube chamber for Neon™ NxT 1-Channel Tubes
③ Low voltage interface port
④ High voltage interface port
⑤ Connector cable
⑥ Low voltage interface connector
⑦ High voltage interface connector

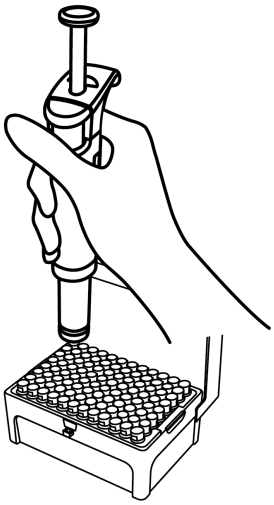
Neon™ NxT 1-Channel Pipette

The Neon™ NxT 1-Channel Pipette utilizes a positive displacement pipette mechanism for pipetting mixtures containing cells and payload (such as nucleic acid). The Neon™ NxT 1-Channel Pipette is a fixed volume pipette and permanently calibrated at the manufacturing stage and does not require any further calibration.

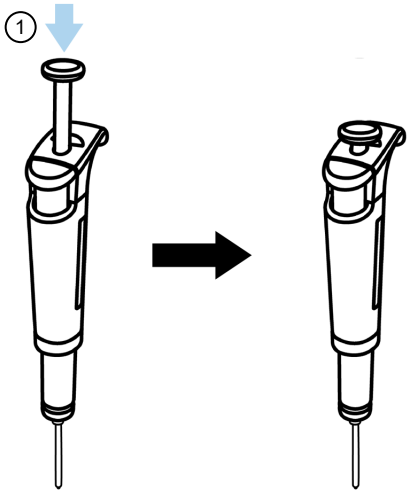
The Neon™ NxT 1-Channel Pipette is designed for use with Neon™ NxT Tips only. Do not use any other tips with the Neon™ NxT 1-Channel Pipette.

① Plunger
② Tip ejector
③ Tip holder

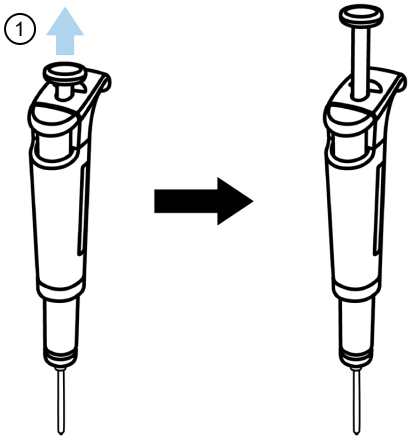
Using the Neon™ NxT 1-Channel Pipette



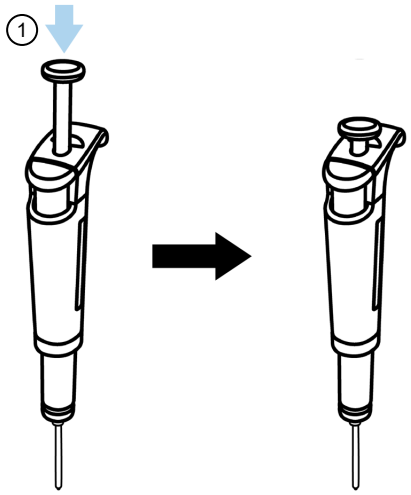
Attach a ClipTip™ by pushing down on a tip until a click is heard.



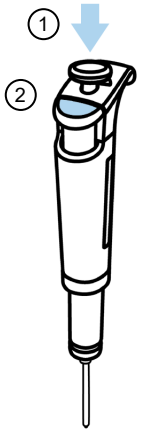
Press the plunger (1) to the second stop to engage the piston (whereupon a click is heard).



Press the plunger (1) to the first stop, then slowly release to retract the piston and aspirate samples.



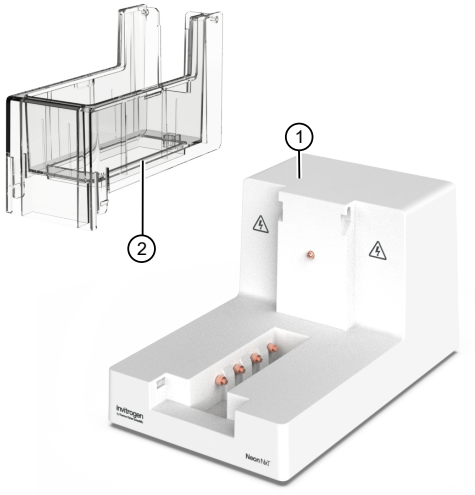
Press the plunger (1) down to the first stop to dispense samples.

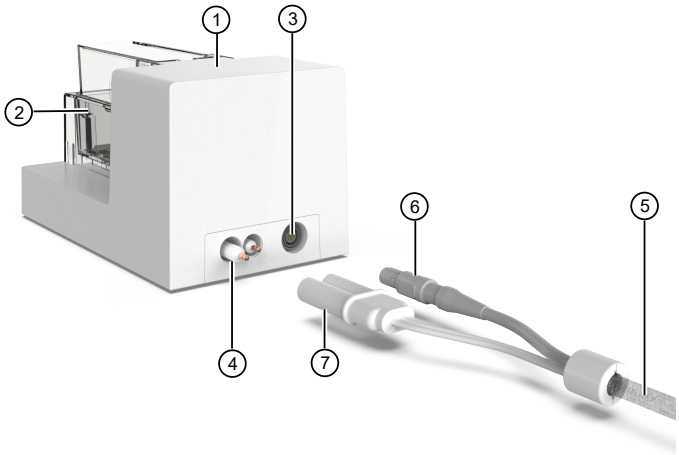


Press the tip ejector (2) to disengage the ClipTip™, then press the plunger (1) to the second stop to release and eject the piston.

Neon™ NxT 8-Channel Pipette Station

The Neon™ NxT 8-Channel Pipette Station holds a Neon™ NxT 8-Channel Pipette during electroporation procedures. The pipette (with attached tips) is held in a Neon™ NxT 8-Channel Tubes which has electrodes near the bottom that transfers the electric field from the electrode inside the Neon™ NxT Tip.





(1) Neon™ NxT 8-Channel Pipette Station
(2) Tube chamber for Neon™ NxT 8-Channel Tubes
(3) Low voltage interface port
(4) High voltage interface port
(5) Connector cable
(6) Low voltage interface connector
(7) High voltage interface connector

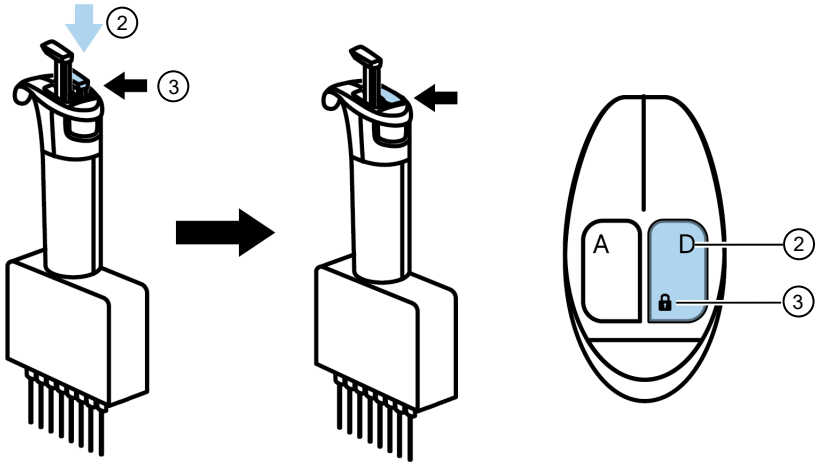
Neon™ NxT 8-Channel Pipette

The Neon™ NxT 8-Channel Pipette utilizes a positive displacement pipette mechanism for pipetting mixtures containing cells and payload (such as nucleic acid). The Neon™ NxT 8-Channel Pipette is a fixed volume pipette and permanently calibrated at the manufacturing stage and does not require any further calibration.

The Neon™ NxT 8-Channel Pipette is designed for use with Neon™ NxT Tips only. Do not use any other tips with the Neon™ NxT 8-Channel Pipette.

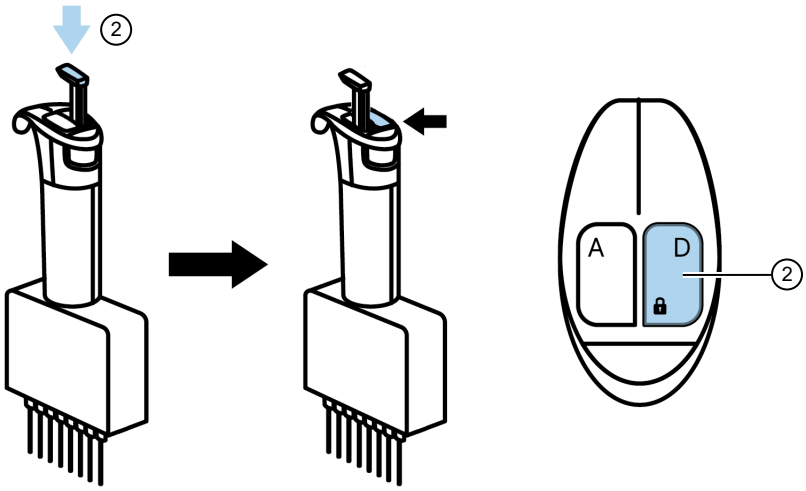
① Aspirate plunger (raised position)
② Dispense plunger (released position)
③ Tip ejector
④ Tip holder

Using the Neon™ NxT 8-Channel Pipette



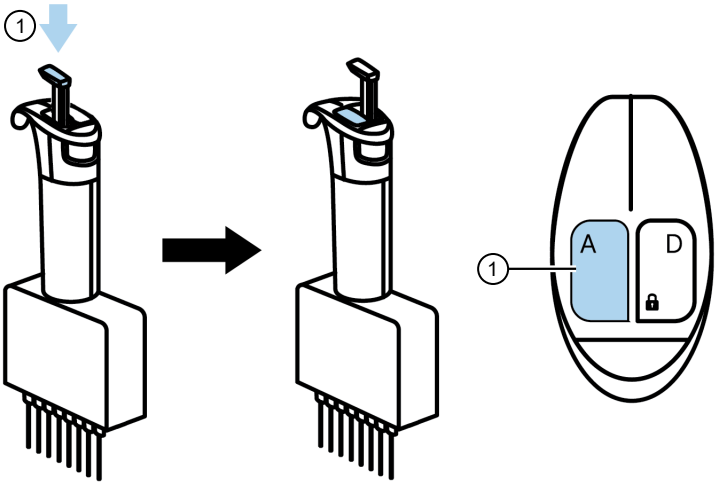
When the Dispense plunger ② is pressed to the first stop, it functions as the Piston engagement button ③.

Note: The Piston engagement button ③ needs to be released before attaching tips (This is done by pressing the Eject button).



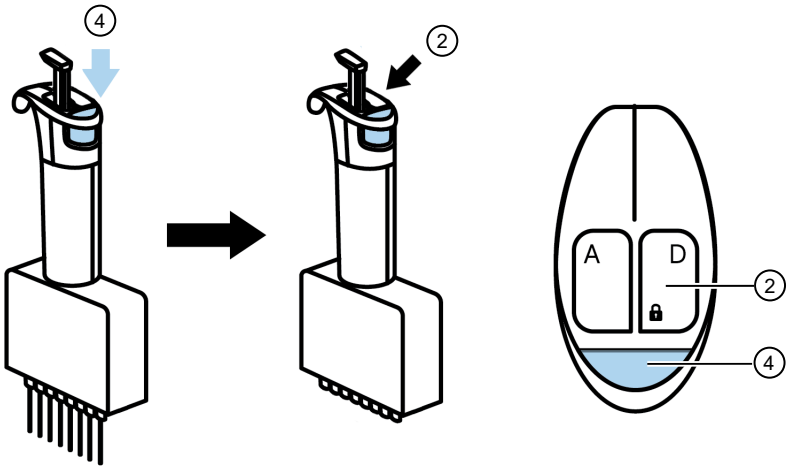
Press the Dispense plunger ② until it is flush with the top of the pipette to:

- Engage the pipette with the pistons in the tips.
- Dispense samples.



Press the Aspirate plunger ① to:

- Retract pistons.
- Aspirate samples.



Press the Eject button ④ to:

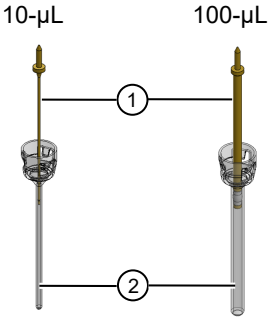
- Eject the tips.
- Release the piston engagement button (when the Dispense plunger ② is all the way down and flush with the top of the pipette).

Neon™ NxT Kit

Neon™ NxT Kits contain Neon™ NxT Tips, Neon™ NxT Tubes, and buffers for electroporation. Neon™ NxT Kits are available in two formats for electroporation of 10 µL or 100 µL samples, in three reactions sizes of 25 × 2 reactions, 96 × 2 reactions, and 384 × 2 reactions (See page 85 for ordering information).

Neon™ NxT Tip

Neon™ NxT Tips are disposable tips composed of a tip and piston used with the Neon™ NxT 1-Channel Pipette. Neon™ NxT Tips contain a gold-plated electrode to create a disposable electric chamber for the delivery of a high electric field to biological samples. The tips are supplied with Neon™ NxT Kits in two formats to support operating volumes of 10 µL and 100 µL, respectively (see page 85 for ordering information).



① Gold plated piston (raised out of tip housing)
② Tip housing

To ensure repeatability and eliminate variation of the transfection conditions within or between experiments, do not use the tip more than 2 times. Oxide formation at the piston surface area can be generated if the tips are used more than 2 times, which decreases electrode function of the piston. Change the tip when switching to a different payload or cell type.

Tip specifications:

Material (tip housing): Polypropylene

Capacity: 10 µL or 100 µL

Neon™ NxT 1-Channel Tubes

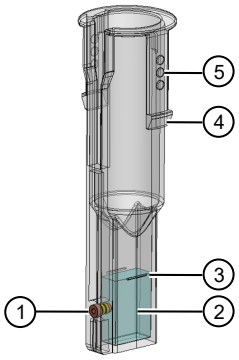
Neon™ NxT 1-Channel Tubes hold electrolytic buffer during electroporation and are inserted into the Neon™ NxT 1-Channel Pipette Station. The Neon™ NxT 1-Channel Pipette with a Neon™ NxT Tip is then inserted into the Neon™ NxT Tube which has an electrode near the bottom that transfers the electric field from the electrode inside the Neon™ NxT Tip. Neon™ NxT Tubes are supplied with Neon™ NxT Kits as well as available separately (see page 85).

To avoid contamination, do not use tubes more than 12 times. Change the tube and buffer when switching to a different payload or cell type.

Tube specifications:

Material: Polycarbonate

Capacity: 2–3 mL



① Electrode
② Buffer
③ Buffer level indicator line
④ Hook profile
⑤ Raised dots to assist grip

Neon™ NxT 8-Channel Tubes

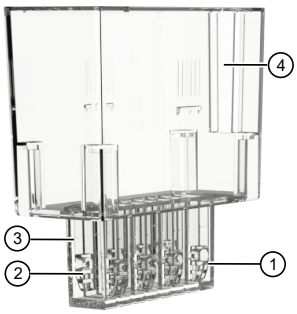
Neon™ NxT 8-Channel Tubes hold electrolytic buffer during electroporation and are inserted into the Neon™ NxT 8-Channel Pipette Station. The Neon™ NxT 8-Channel Pipette with Neon™ NxT Tips are then inserted into the Neon™ NxT Tube which has electrodes near the bottom that transfers the electric field from the electrode inside the Neon™ NxT Tip. Neon™ NxT Tubes are supplied with Neon™ NxT Kits as well as available separately (see page 85).

To avoid contamination, do not use tubes more than 12 times. Change the tube and buffer when switching to a different payload or cell type.

Tube specifications:

Material: Polycarbonate

Capacity: 2–3 mL



① Electrode
② Individual tip buffer chambers
③ Buffer level indicator line
④ Hook for tube chamber

Upon receiving the device

Examine the unit carefully for any damage incurred during transit. Any damage claims must be filed with the carrier. If the instrument is damaged during shipment, contact Technical Support for assistance. To register the device, activate your warranty, and be notified of important updates, go to **thermofisher.com**.

Unpacking instructions

Consult the following instructions to unpack the Neon™ NxT Electroporation System. The weight of the system is 13 pounds (5.9 kg).

1. Open the shipping box to unpack the Neon™ NxT device. Save the box and other packaging material (in case you need to transport or ship the unit).
2. Remove the packing material, then inspect the Neon™ NxT device and Neon™ NxT 1-Channel Pipette Station for shipping damage.
3. Remove the Neon™ NxT device and the Neon™ NxT 1-Channel Pipette Station from the box and place them on a flat, level surface.
4. Set up the Neon™ NxT Electroporation System as described on page 20.

Getting started

Set up the Neon™ NxT Electroporation System

1. Unpack the Neon™ NxT Electroporation System as described in “Unpacking instructions” on page 20.
2. Place the Neon™ NxT device on a level laboratory bench. Keep an area of free space at least 10 cm surrounding the unit to ensure proper ventilation.

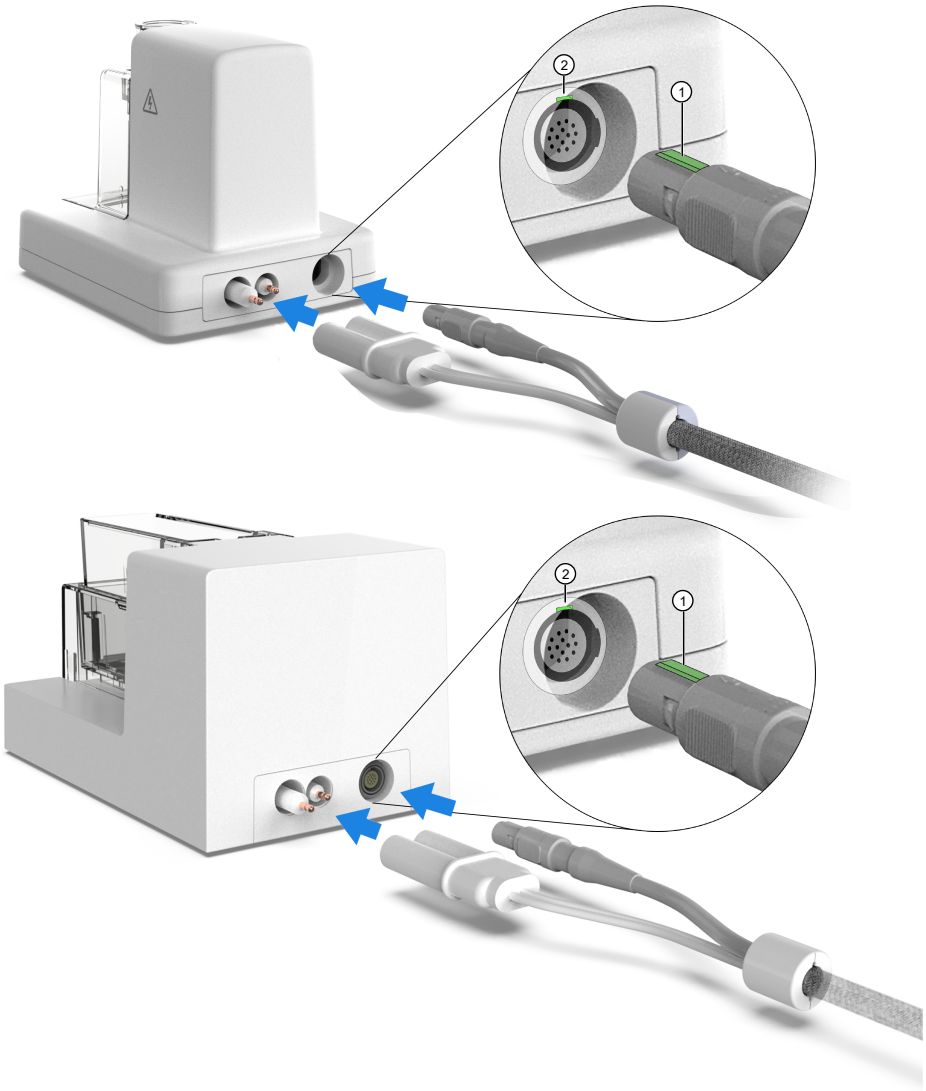
   Note: The Neon™ NxT device has a small footprint and can be easily set up in the tissue culture hood for convenience.

   IMPORTANT! Position the device properly such that the **power** switch and AC inlet located at the rear of the unit (see “Front and rear view” on page 11) are easily accessible. Be sure to position the device such that it is easy to disconnect the unit.

   Since Neon™ NxT device is air-cooled, its surface may become hot during operation. When installing the device, leave a space of more than 10 cm from the back of the device.
3. Place the Neon™ NxT Pipette Station near the Neon™ NxT device.

4. Connect the high voltage and low voltage interface connectors on the cable to the high voltage and low voltage interface ports at the rear of the Neon™ NxT Pipette Station.

Note: To avoid damaging the cable connector, ensure the notch on the low voltage connector is aligned with the corresponding slot on the low voltage port.



① Notch on low voltage connector
② Slot on low voltage port

IMPORTANT! Always handle the connectors using the cord plug and not the cord cable when connecting or disconnecting the connectors.

5. Connect the high voltage and low voltage interface connectors on the Neon™ NxT Pipette Station to the high voltage and low voltage interface ports at the rear of the Neon™ NxT device.

Note: To avoid damaging the cable connector, ensure the notch on the low voltage connector is aligned with the corresponding slot on the low voltage port.

Ensure the notch on the connector of the cable is aligned with the corresponding slot on the port of the Neon™ NxT device.

IMPORTANT! Always handle the connectors using the cord plug and not the cord cable when connecting or disconnecting the connectors.

6. Ensure the AC power switch is in the **Off** position (see “Front and rear view” on page 11).

7. Attach the power cord to the AC inlet at the rear of the Neon™ NxT device, then plug the cord into an electrical outlet. Use only properly grounded AC outlets and power cords.

The instrument operates at voltages of 100–240 VAC and the frequency range of 50/60 Hz. Ensure that the local supply voltage in the laboratory conforms to that specified on the type label on the back of the instrument

8. Press the main power switch at the rear of the device to the **ON** position to turn on the power.

9. The Neon™ NxT device is operated by the digital touch screen at the front of the device. See “User interface overview” on page 24 for details.

After the Neon™ NxT Electroporation System is set up, see “Using the Neon™ NxT Electroporation System” on page 49 for instructions on performing electroporation.

Exchange a Neon™ NxT Pipette Station

1. Disconnect the high and low voltage connectors of the existing Neon™ NxT Pipette Station from the back of the Neon™ NxT device.

2. Set up the new Neon™ NxT Pipette Station to the Neon™ NxT device as described in “Set up the Neon™ NxT Electroporation System”.

User interface overview

Symbol	Function
Main dial	
	• **Set up run** • Select a protocol or plate map • Create a new protocol (see “Create or edit protocols” on page 38 for more details) • Create a new plate map (see “Create or edit plate maps” on page 40 for more details) Displays the instrument status when a protocol is run (see “Touchscreen status indicators” on page 25)
Optimization screen	
	• View protocols • Create a new protocol (see “Create or edit protocols” on page 38 for more details) • Edit an existing protocol (see “Create or edit protocols” on page 38 for more details)
Create protocol screen	
	• Calculate volume for protocol (see “Calculate electroporation volumes for cell suspension” on page 50 for more details)

Touchscreen controls

Table 3 General touchscreen controls

Button	Function
	Returns to the previous screen.
	Go to **Home** screen.
	Go to **Sign in** screen.
	Go to **Settings** screen.
	Close the current modal window.

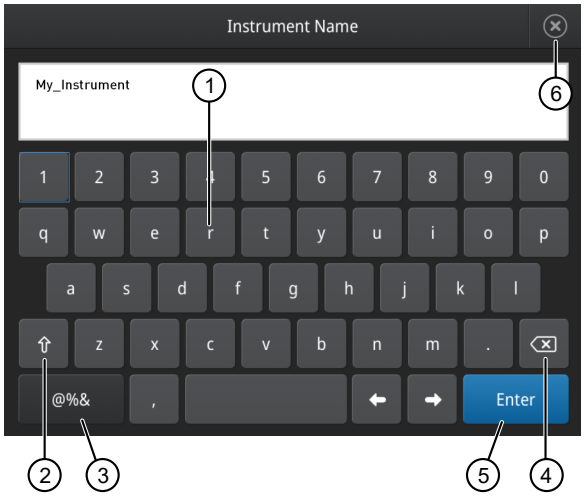
Touchscreen status indicators

Table 4 Status indicators

Button	Function
	Indicates whether a USB device is inserted into the instrument.
	Indicates whether the Wi-Fi is on or off.
	Indicates whether the instrument is connected to wired network.
	Indicates whether the instrument is connected to the Thermo Fisher™ Connect Platform.

Enter text

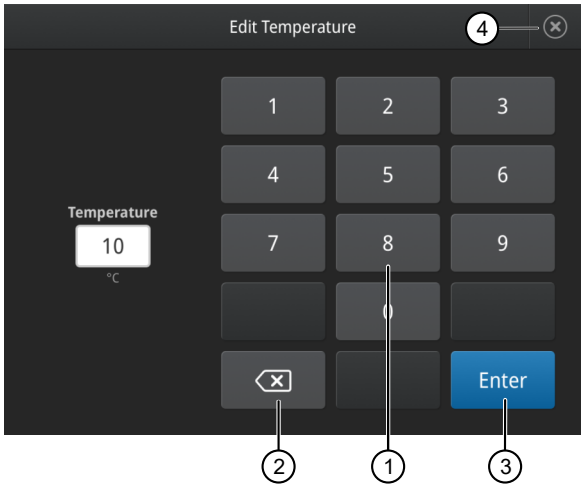
When you press a field that requires the input of text, the text editor, as seen in the following figure, opens.



① Enter a letter
② Change letter case
③ Enter punctuation or other symbols
④ Delete
⑤ Close and save
⑥ Close without saving

Enter numbers

When you press a field that requires a numerical input, the numeric editor, as seen in the following figure, opens.



① Enter a number
② Delete/backspace
③ Close and save
④ Close without saving

Connect the instrument to the Internet

The instrument can connect to the Internet by either wired or wireless methods.

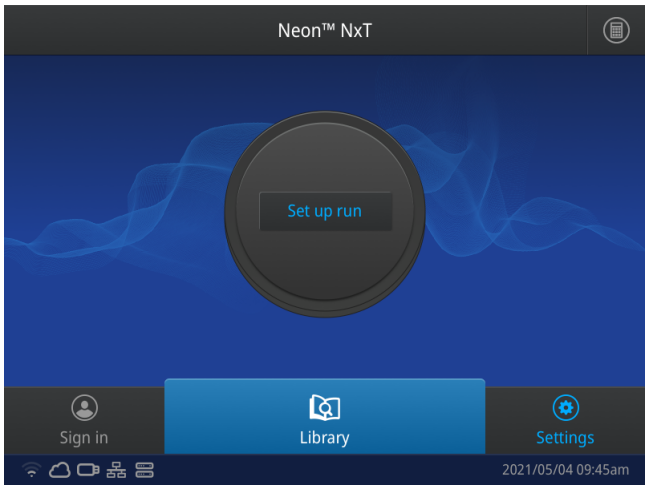
To connect by wired method through the instrument Ethernet port using a cable, see “Set up a wired connection” on page 27.

To connect by wireless method through the instrument USB wireless adapter port with a USB-enabled Wi-Fi card, see “Set up a wireless connection” on page 30.

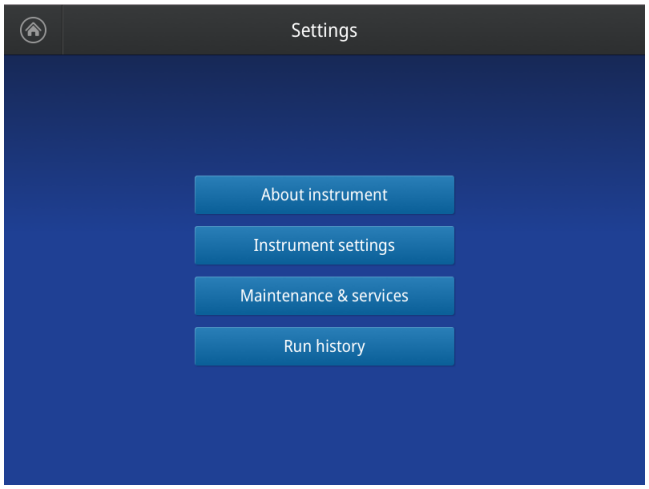
Set up a wired connection

Connect one end of a Ethernet cable to the instrument Ethernet port, and the other end to an Ethernet port wall plug (see “Front and rear view” on page 11 for port location).

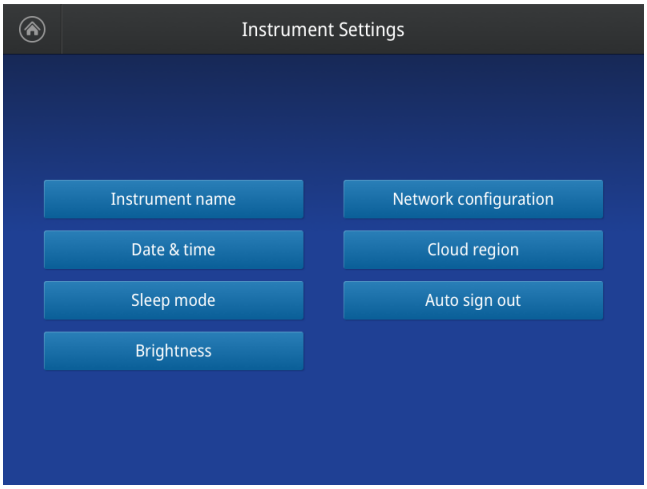
1. On the **Home** screen, select **(Settings)**.



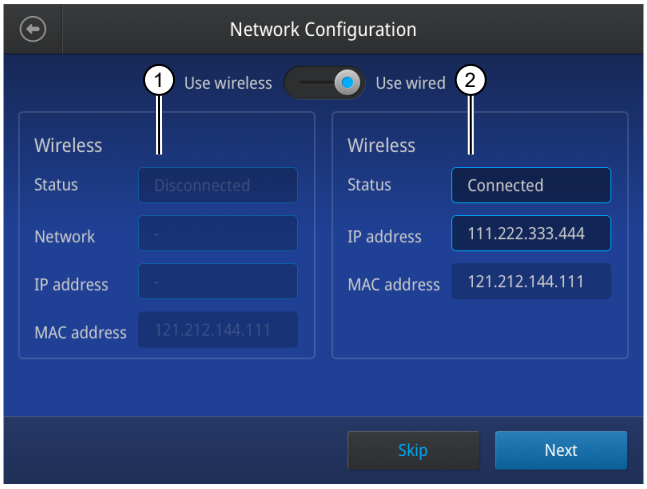
2. In the **Settings** screen, select **Instrument Settings**.



3. In the **Instrument Settings** screen, select **Network configuration**.



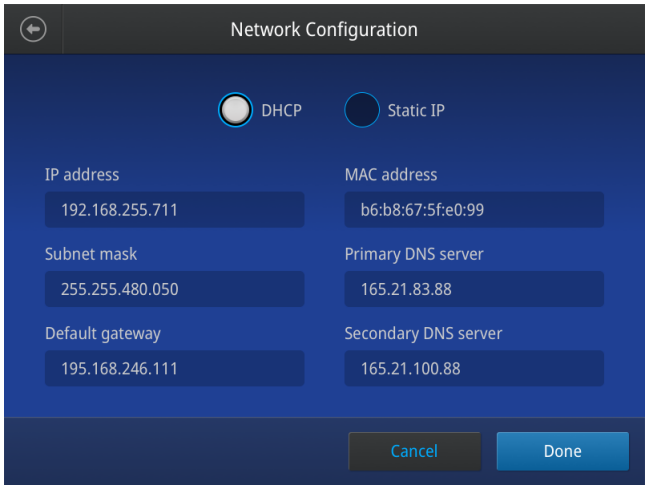
4. In the **Network Connection** screen, select a field in the **Wired** panel.



① Wireless panel ② Wired panel

5. Select a method to enter an IP address.
   a. Select **DHCP** to obtain an IP address automatically. A check mark appears when DHCP is selected.

b. Select **Static IP** to enter an IP address manually, then enter the appropriate IP addresses for the instrument, the Subnet Mask, and, optionally, the Default Gateway, the Primary DNS Server, and the Secondary DNS Server using the numeric editor. Addresses are in the form of X.X.X.X, where each X is a 3- digit number, from 001 to 255.



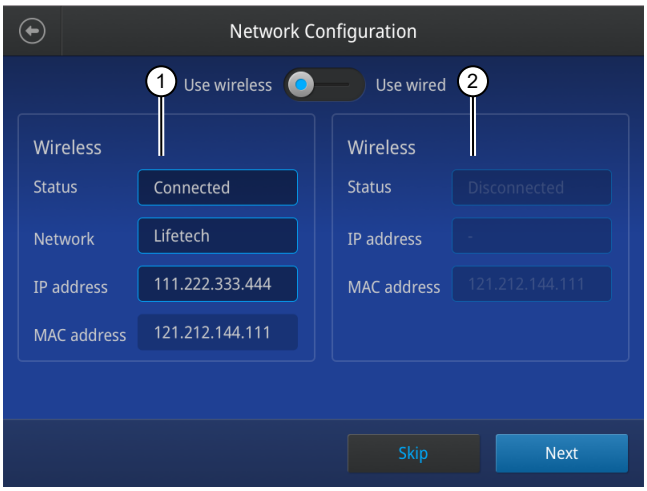
Note: If your instrument is not on a network, you do not need to set the IP address. Ask your system administrator if the IP address is assigned statically or dynamically. For static addresses, you need to know the IP address for the instrument, the subnet mask, and the default gateway.

6. Select **Done** to save the changes and go back to the **Instrument Settings** screen or select **Cancel** to exit the screen without saving the changes.

Set up a wireless connection

Connect the High-Power USB Wi-Fi Module (Cat. No. A26774 to the USB wireless adapter port (see "Front and rear view" on page 11 for port location).

1. See "Set up a wired connection" on page 27 Steps 1 through 3 to find the **Network configuration** screen.
2. In the **Network configuration** screen, select a field in the **Wireless** panel.



① Wireless panel ② Wired panel

Note: During initial setup, if you selected the Wired option in the **Network Connection** screen, you will be required to enter the IP address if you selected the Static IP wired option. If you selected the Dynamic IP wired option, the IP address is automatically populated.

3. Once a wireless connection has been detected, a list of the available networks is displayed. Select the network name of your choice or select **Join others**.

Note: If you choose **Join others**, the **Configure and Join Network** screen opens.

4. In the **Configure and Join Network** screen, select the **Network Name** field, then enter the name and security type of the network.
5. Select the security type from the **Security type** dropdown menu.

Note: Contact your IT Systems Administrator for information on security type.

Select from the following options:

- Open
- WEP
- WPA Personal
- WPA2 Personal
- WPA Enterprise
- WPA2 Enterprise

Note: The above options are available only if **Join Other Network** was selected in Step 3. You cannot change the security type if you selected an existing network.

6. Select **Join** to continue or **Cancel** to exit from the **Find and Join a Network** screen.
7. Depending on the security type you have selected, enter the appropriate passwords and select **Join**.
8. If all the entered information is correct, the **Network Connection Complete** screen will appear. Select **OK** to continue.

Note: If incorrect information was entered the **Network Connection Failed** screen will open. Select **OK** to continue to the **Security type** screen.

Create a user profile on the instrument

1. Select **(Sign In)** > **Get started** > **Create profile**.
2. Fill in the required text fields and enter a four digit PIN to create your user profile.

Note: The first profile created is automatically given an Administrator profile (indicated by an asterisk after the **Username**).

3. Select **Create**.

Manage user profiles

All users can manage their profiles to edit personal folder names, change PINs, and link to the cloud by selecting their **(Profile)** to enter their **My Profile** page.

Users with Administrator profiles (as indicated by an asterisk after their user name) are able to manage all user accounts by selecting **All accounts** after entering their **My Profile** page.

The following actions are available from the user profile screen:

- Change a PIN
- Create a new user profile
- Grant administrator rights to selected user profile (Administrator only)
- Delete a user profile (Administrator only)
- Delete a PIN (Administrator only)

Change a user PIN

1. Select **Edit**.
2. Enter the old PIN.
3. Enter a new four digit PIN.
4. Re-enter the new PIN, then select **Done**.

Delete a user PIN

If a user PIN is forgotten, an administrator can delete the existing PIN to allow a new one to be created. This function resets a PIN, so the user with a deleted PIN is prompted to create a new PIN the next time they sign in)

1. Select **All accounts**.
2. Select the account with the forgotten PIN.
3. Select **Delete PIN**.

Delete a user profile

1. Select **All accounts**.
2. Select the account to be deleted.
3. Select **Delete account**.

Assign or remove administrator privileges

1. Select **All accounts**.
2. Select the account to be modified.
3. Switch the toggle to **Yes** to grant privileges, or **No** to remove privileges.

An asterisk appears next to user profiles with administrator privileges.

About the Thermo Fisher™ Connect Platform

The Thermo Fisher™ Connect Platform enables access to the Neon™ NxT device through InstrumentConnect by way of a web browser or mobile device. This cloud-based tool allows the user to perform the following functions when the instrument has Internet connectivity.

Features

- Monitor real-time instrument status.
- Access the TransfectionLab App to set up experiments.
- Upload and download custom protocols to your Connect account.
- Securely store, access and manage personal protocols and protocols in DataConnect.
- Share protocols within a research team or with colleagues in another laboratory, location, or country.
- Automatically or manually upload run report from the instrument to your Connect account.
- Upgrade instrument software automatically, without hardware or manual updates.

Create a Connect Platform account

1. Go to **thermofisher.com/connect** from your web browser.
2. Click **Sign up now** and follow the prompts to create an account.
   Your e-mail address is used as your username.
3. When signed in, click **Update PIN number**.
4. Enter a PIN number in the **new** and **confirm** fields.
   The PIN number is necessary to sign in to Connect Platform from the instrument.

Create a PIN number

1. Log in to your Connect Platform account using a web browser.
2. Navigate to **(InstrumentConnect)**.
3. Select **Update PIN number**.
4. Confirm the PIN number.

Set up a new administrator

1. To set up a new adminstrator, log in to current administrator Connect Platform account.
2. Select **Instruments**.
3. Select the Neon™ NxT device for the current administrator.
4. Select **Manage users**.
5. Assign the administrator role to another user linked to the same instrument.

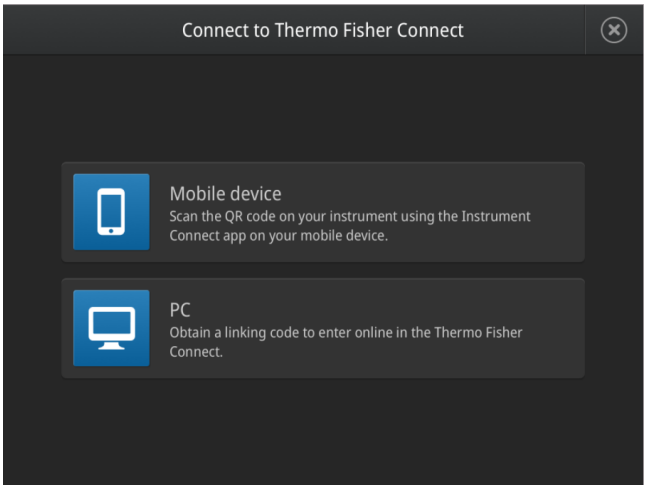
Add an instrument to your Connect Platform account

The Connect Platform supports access to the Neon™ NxT device with the InstrumentConnect application on your mobile device or from a web browser. When the instrument is connected, real-time instrument status can be viewed from the InstrumentConnect application.

IMPORTANT! The first Connect Platform account that links to the instrument becomes Administrator by default. If the first user needs to be unlinked from the instrument, a new user must be assigned the Administrator role beforehand. Failure to do so will result in the loss of instrument connectivity for all other linked users. For instructions on how to setup a new Administrator see “Set up a new administrator” on page 34.

Link the instrument to Connect (Administrator only)

1. Select **(Sign In)** ▸ **Link cloud**, then select the cloud region of the instrument.
2. Select the method for linking the instrument to Connect.



Add an instrument to your Connect account (PC)

1. Log in to your Connect account using a web browser.
2. Select **(InstrumentConnect)** from the left navigation strip.
3. Select **(Add an Instrument)** from the top navigation strip.
4. Select **Neon™ NxT** from the **Instrument type** drop down menu, then click **Next**.
5. Enter the linking code generated by the instrument in the text box, then click **Send**.
   Upon successful authentication, the instrument is linked to Connect.

Add an instrument to your Connect account with QR code (mobile device)

Install a QR code scanner app on your mobile device to connect to the instrument using the QR code.

1. Open the InstrumentConnect application on a mobile device.
2. Select **QR code**.
3. Take a picture of the QR code on the **Notifications** screen of the instrument with your mobile device.

8-Channel Software Activation

8-Channel Software Activation is required to use the Neon™ NxT Electroporation System 8-Channel Upgrade Package with the Neon™ NxT Electroporation System with 1-Channel Pipette (Cat. No. NEON1S, NEON1SK).

Note: This is a one time activation procedure, and firmware updates or factory resets will not require re-activation to be performed.

Activate 8-Channel Software

1. Login to your Connect account to access the TransfectionLab App at **apps.thermofisher.com/apps/transfectionconnect**.
2. Select **Create experiment ▸ 8-Channel Software Activation**.
3. Enter the order number for the Neon™ NxT Electroporation System 8-Channel Upgrade Package.
4. Enter the serial number for the Neon™ NxT device.
   The serial number can be found at the top of the **Home** screen, on the **About Instrument** screen, or on the rear panel of the Neon™ NxT device.
5. After the required information is verified, select **Get Activation File** to download the license.
   A copy of the license is also sent to the email address associated with your Connect account.

6. Transfer the license to a USB drive.

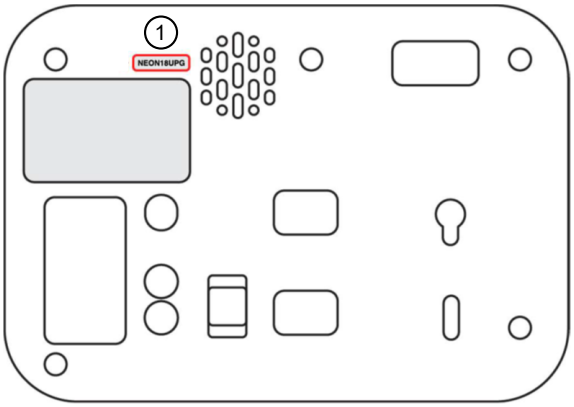
   Note: The license should be saved in the root directory of the USB drive, and not in a folder or subdirectory.

7. Select **Settings** ▸ **Maintenance & Servies** ▸ **License upgrade**.

8. Insert the USB drive into the Neon™ NxT device and select **Next**.

9. Select the license file, then select **Activate**.

10. Place the NEON18UPG sticker from the notification included with the Neon™ NxT Electroporation System 8-Channel Upgrade Package onto the back of the instrument.



① Place NEON18UPG sticker here.

2 Methods

Electroporation protocol options

If a protocol with the necessary electroporation parameters for your cell type already exists, the protocol can be chosen from the **Protocol Library** by selecting **Library ▸ Protocol Library**.

- The **Protocol Library** contains a list of all available protocols stored on the instrument. Swipe up or down in the list pane to scroll through the protocols.
- Protocols can also be filtered by selecting the categories **User created**, **Cell Specific**, or **Optimization**.
  - Select **Optimization** to find an optimization protocol for a new cell type without specific electroporation parameters, (see “Optimization protocol” on page 73).
  - Select **Cell Specific** to see a list of protocols that have a defined cell type.
  - Select **User created** to see a list of protocols created by instrument users.

If a new protocol is required, there are two methods for creating a electroporation protocol:

- Use **Quick Run** to create a new protocol (see “Create or edit protocols” on page 38 for details).
- Use **Quick Run** to edit an existing protocol (see “Create or edit protocols” on page 38 for details).

If a plate map is already set up for electroporation, the plate map can be chosen from the **Plate Library** by selecting **Library ▸ Plate Library**.

- Select **Optimization** to find a plate map for a new cell type without specific electroporation parameters, (see “Optimization protocol” on page 73).
- Select **User created** to see a list of plate maps created by instrument users.

Electroporation parameters

The Neon™ NxT Electroporation System is designed to operate within specific parameters. The values and limits for each parameter are listed below.

Parameters that can be modified include:

- **Pulse voltage** (range: 500–2,500 V)
- **Pulse width** (range: 1–100 ms)
- **Number of Pulses** (range: 1–10 pulses)
- **Cell Type**
- **Buffer Type** (Resuspension R Buffer, Resuspension T Buffer, or Resuspension Genome Editing Buffer)
- **Payload Type**

The individual parameters (voltage, pulse width, pulse number) have their own independent upper limits. However, some combinations of these variables can exceed the energy limit of the system even if still within the individual limit. For example, a protocol of 2300 V/30 ms/3-pulses has all variables within acceptable limits, but cannot be run as it exceeds the energy limit of the system.

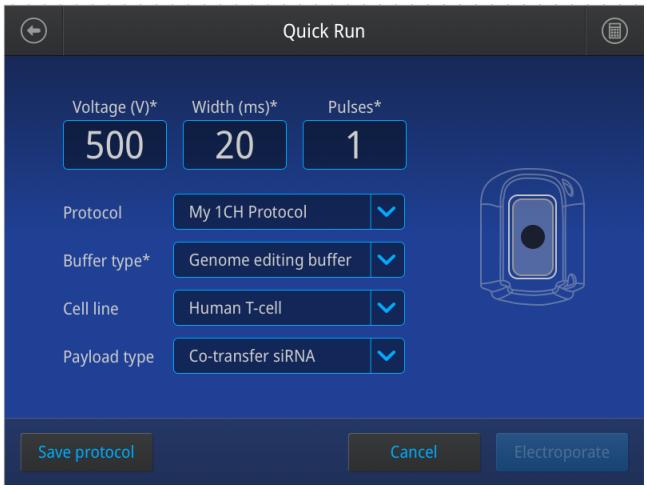
Note: There are two energy level limits.

- Arcing limit: If the limit is exceed, a warning that arcing may occur is given, but the user can still run the protocol.
- Hardware limit: If the limit is exceed, the user cannot run the protocol.

Create or edit protocols

Create a protocol

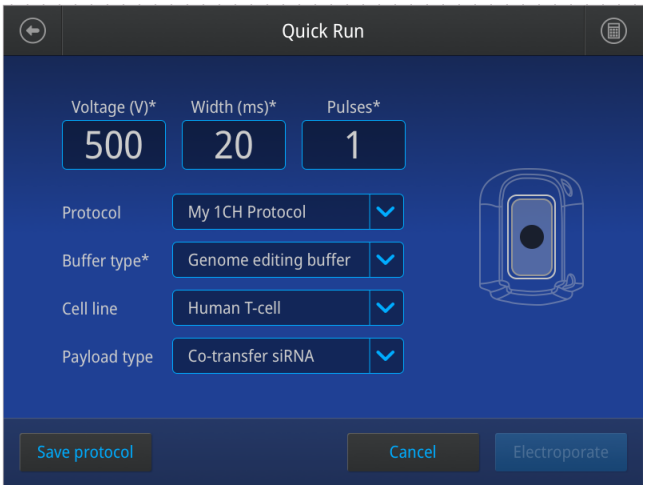
1. In the **Home** screen, select **Set up run** ▸ **Quick run**.
2. Select a text field or open a dropdown menu to set the electroporation parameters (voltage, width, pulses, buffer type, cell line, payload) for the protocol.



3. Select **Save protocol** (see “Save a Protocol” on page 39), **Electroporate** to run the protocol, or **Cancel**.

Edit a protocol

1. In the **Home** screen, select **Set up run** ▸ **Quick run**, or select **Library**.
2. Select a text field or open a dropdown menu to set the electroporation parameters for the protocol.



3. Select **Save protocol** (see "Save a Protocol" on page 39), **Electroporate** to run the protocol, or **Cancel**.

Save a Protocol

1. Once edits to a protocol are complete, select **Save** to save the protocol.
2. In the **Save** screen, enter a name for the edited protocol.

Characters allowed	Characters not allowed
<100 characters	>100 characters
Letters, numbers, spaces, underscores, and dashes	% * ? | ; : , ! @ # $. ( ) < > / \ " ' ` ~ { } [ ] = + & ^ (tab)

3. Select **Save**.

Create or edit plate maps

Plate maps are used to plan out how cells are plated after electroportation. Fill the wells where cells will be plated with the appropriate cell culture media.

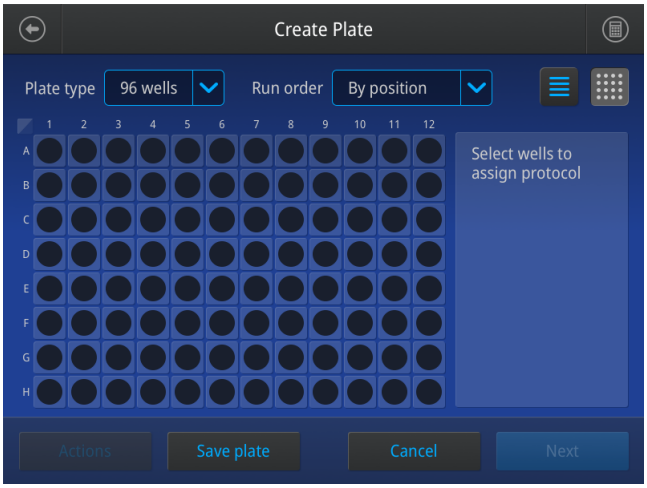
Create a plate map

A plate map is created from the plate view. Once protocols have been assigned to the wells, details can be viewed from the list view.

1. In the **Home** screen, select **Set up run** ▸ **Create plate**.
2. Select the plate type (6-well,12-well, 24-well, 96-well).

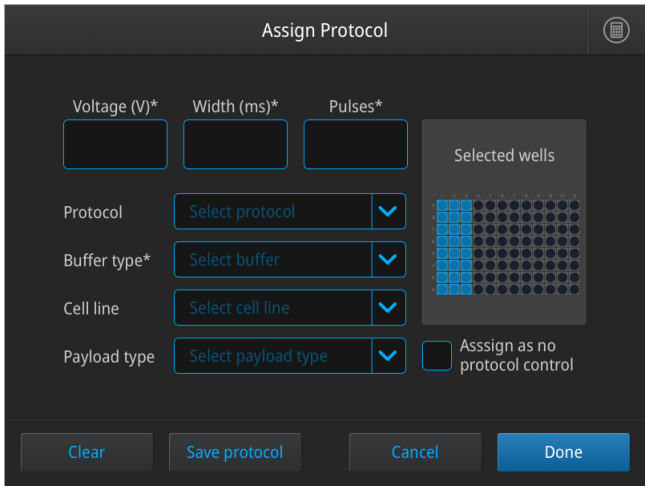
   Note: The 8-channel system can only be used with 24-well and 96-well plates.

3. Select the run order for the wells (from top to bottom, or in order of increasing voltage).
4. Select wells.
   a. Tap a well on the plate map to make a selection.
   b. Tap a selected well on the plate map to undo a selection.



5. Select **Assign protocol**, then choose a protocol or create a protocol to electroporate the cells assigned to those wells.
   a. Select **Save protocol** (see “Save a Protocol” on page 39) to save newly entered electroporation parameters.
   b. Select **Clear** to clear electroporation parameters.
   c. Select **Cancel** to return to the plate map without assigning a protocol to the selected wells.

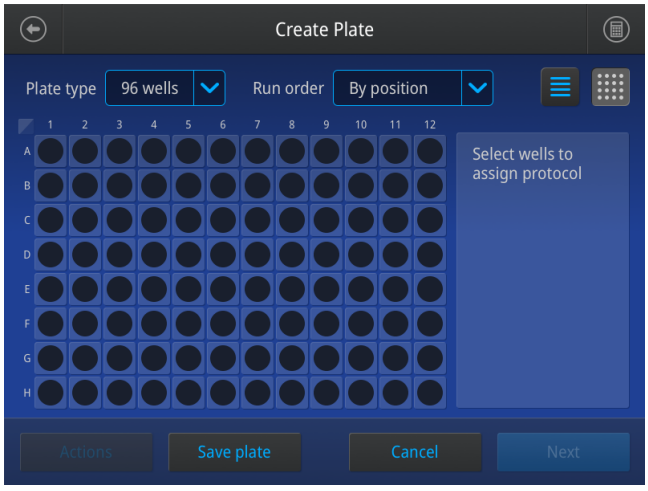
d. Select **Done** to assign a protocol to the selected wells.



6. Select **Save plate** and proceed to electroporation, or perform other actions.
   a. Select **Save plate** (see “Save a plate map” on page 42).
   b. Select **Next** to proceed to electroporation.
   c. Select **Cancel** to cancel the entire workflow.
   d. Select **Actions** to save unsaved protocols or plate details.

Edit a plate map

1. In the **Home** screen, select **Set up run** ▸ **Open plate**.
2. Select wells.



3. Assign the protocol used to electroporate the cells assigned to those wells.

4. Select **Save protocol** (see “Save a Protocol” on page 39), **Clear**,**Cancel**, or **Done**.

5. Select **Save plate** (see “Save a Protocol” on page 39), **Next**, **Cancel**, or **Actions**.

Save a plate map

1. Once edits to a protocol are complete, select **Save** to save the plate map.

2. In the **Save** screen, enter a name for the edited protocol.

Characters allowed	Characters not allowed
<100 characters	>100 characters
Letters, numbers, spaces, underscores, and dashes	% * ? | ; : , ! @ # $. ( ) < > / \ " ' ` ~ { } [ ] = + & ^ (tab)

3. Select **Save**.

Manage protocols

Manage protocols from the protocol selection page by selecting **Actions ▸ Manage Protocol**.

From the Manage Protocol page, the following features are available:

- Examine protocol details
- Delete protocols
- Export protocols
- Filter protocols by name, user, cell type, or date

Import or export protocols

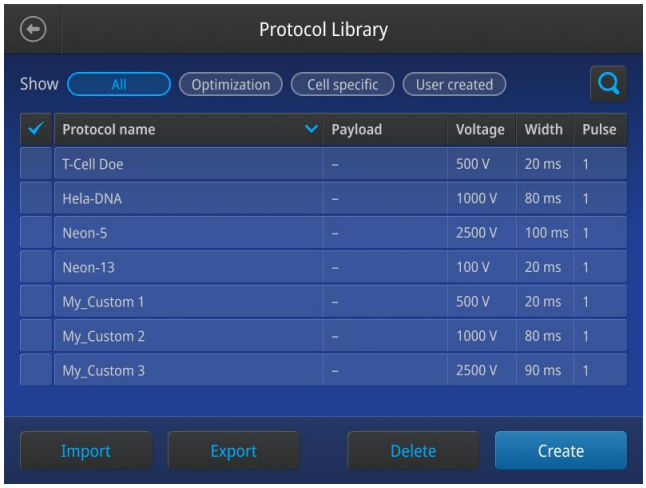
Import or export protocols and plate maps from the protocol library by selecting **Actions**.

Protocols can be imported or exported from the following locations:

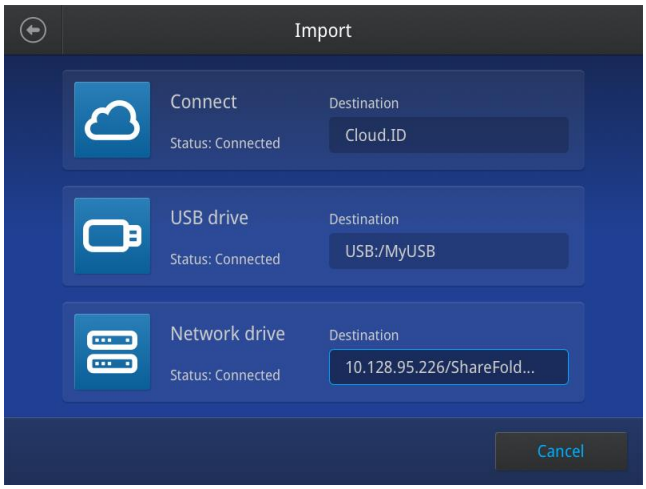
- TransfectionLab app on the Thermo Fisher™ Connect Platform (see “About the Thermo Fisher™ Connect Platform” on page 33 for details)
- USB memory device
- Network drive

Import a protocol

1. Select **Library** ▸ **Import**.

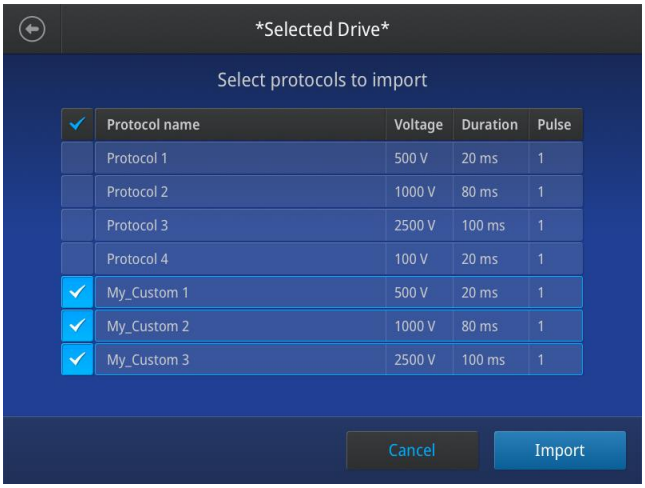


2. Select the location from which the files will be imported (Thermo Fisher™ Connect Platform, USB drive, network drive)



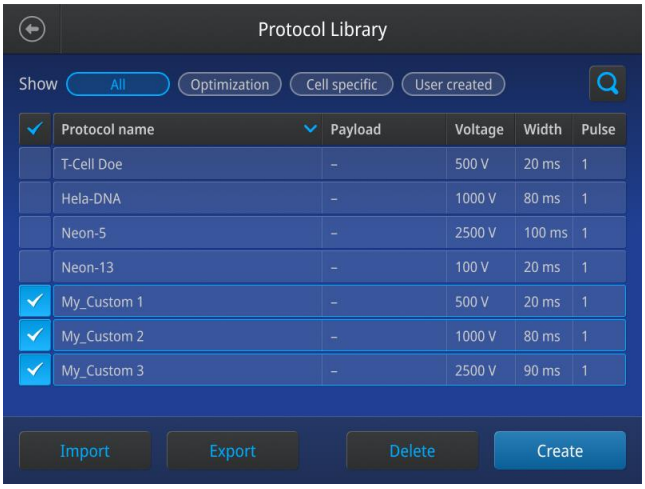
Note: To make a network drive connection, see “Set up or change a network drive connection” on page 45.

3. Select the protocols to import, then select **Import**.

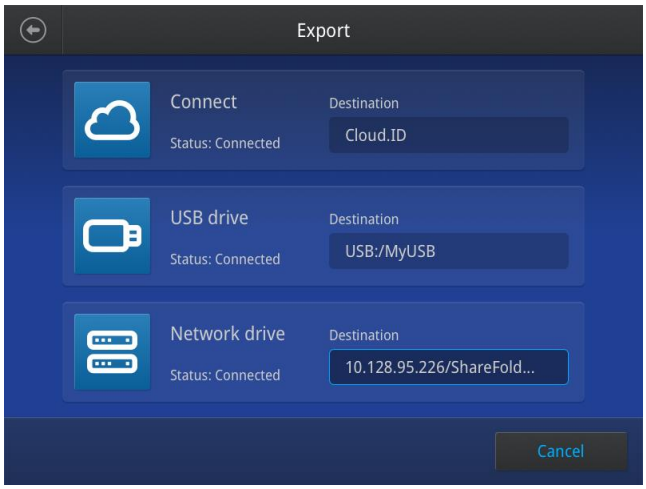


Export a protocol

1. Select **Library** ▸ **Protocol Library**, then select the protocols to be exported.



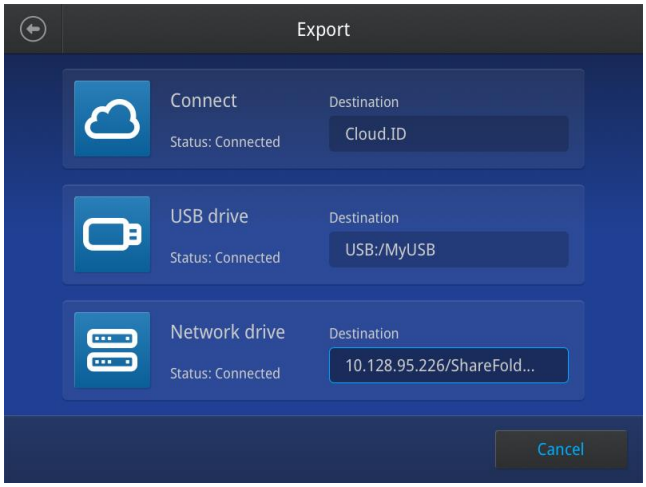
2. Select **Export**, then select the destination for the files to be exported (Thermo Fisher™ Connect Platform, USB drive, network drive).



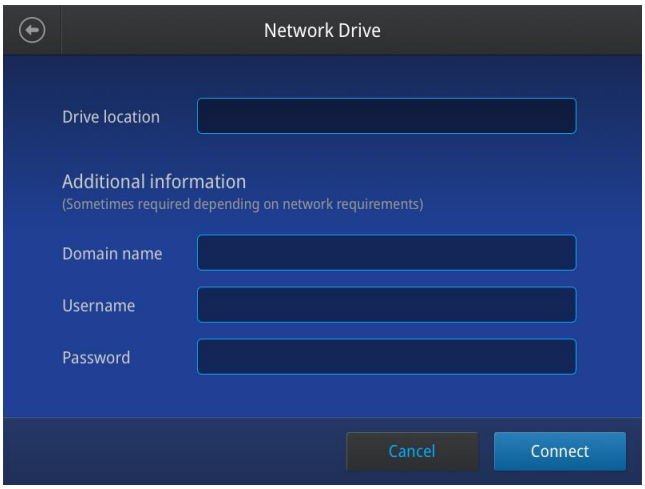
Note: To make a network drive connection, see “Set up or change a network drive connection” on page 45.

Set up or change a network drive connection

1. Select the **Network drive Destination** field.



2. Enter the network drive details (drive location, domain name, user name, password) in the **Network Drive** screen, then select **Connect**.



General guidelines

Guidelines for using kits

To use the Neon™ NxT device for electroporation of mammalian cells, you need to purchase the Neon™ NxT Kits. Ordering information is on page 85. **Do not** use any other kits with the unit.

Note: To obtain the best results, follow these recommendations:

- Based on your initial results, you may need to optimize the electroporation parameters for your cell type and DNA/siRNA. A preprogrammed 24-well optimization protocol is included in the device for your convenience.
- Before using the device with your samples, ensure that you are able to insert and use the Neon™ NxT Pipette and Tip correctly (see “Using the Neon™ NxT 1-Channel Pipette” on page 14 or “Using the Neon™ NxT 8-Channel Pipette” on page 16), as well as into the Neon™ NxT Pipette Station (see page 49 for details).
- Wear gloves, laboratory coat, and safety glasses during electroporation.
- Always use the Neon™ NxT device with Neon™ NxT Kits for electroporation of mammalian cells.
- The Neon™ NxT Electroporation System is compatible for use with most mammalian cells including primary and stem cells.
- **Use high quality payloads (such as DNA, siRNA, RNP) to obtain good transfection efficiency.**
- Follow the guidelines on page 52–53 for cell preparation.
- Use an appropriate GFP (green fluorescent protein) construct (see page 48 for details) or payload controls to determine transfection efficiency.
- Discard the Neon™ NxT Tips after 2 usages and Neon™ NxT Tubes after 12 usages as a biological hazard. We strongly recommend changing tube and buffer when switching to a different payload (plasmid DNA/siRNA/RNP) or cell type.

- Visit **thermofisher.com/transfectionprotocolsandcitations** for a library of electroporation protocols for commonly used cell types. These protocols are also stored on the instrument. Ensure the instrument has the latest version of the software for access to the most current protocols.

Guidelines for selecting buffers

There are different cell resuspension buffers available for use with the Neon™ NxT Electroporation System. Select the appropriate resuspension buffer based on application and following guidance. Optimization between the three buffers is recommended for best results.

- Neon™ NxT Resuspension R Buffer is recommended as the standard resuspension buffer for gentle electroporation with most cell types and payload types.
- Neon™ NxT Resuspension T Buffer is recommended under the following conditions:
  - High voltage protocols of 1900V or more
  - Difficult to transfect cell types such as naïve or resting T-cells
  - Heat sensitive cell types
  - Larger payload transfection
- Neon™ NxT Resuspension Genome Editing Buffer is specifically designed for knock-in (KI) applications in genome editing.
- Neon™ NxT Electrolytic E10 Buffer is used in buffer tubes for 10 µL reactions.
- Neon™ NxT Electrolytic E100 Buffer is used in buffer tubes for 100 µL reactions.

Guidelines for electroporation vessels

Pre-electroporation vessels are used to hold samples to load the Neon™ NxT Pipette for electroporation. Regardless of the loading vessel, it is important to ensure that there is enough sample so that the bottom of the pipette tip is completely submerged, preventing the formation of bubbles.

- For 8-channel electroporation, sterile 96-well plates, PCR tubes, or reagent reservoirs can be used.
- V-bottom or U-bottom 96-well plates can be used for 8-channel 10 µL reactions. However, they may not be appropriate for 100 µL reactions as the pipette tip may not reach the bottom of the plate.
- Flat-bottom 96-well plates are recommended for both tip types where the plate can be tilted.

Post-electroporation vessels are used for seeding cells after electroporation. When seeding cells, it is important to achieve the proper cell concentration. Seeding cells at densities that are either too concentrated or too dilute may not yield optimal results. In addition, some cells are sensitive to hypoxia, and their growth may be affected if the depth of the media is too high (e.g., if using deep well plates).

- The most suitable vessel for post-electroporation cell seeding in 8-channel electroporation is 96-well plate. If necessary, cells can be further diluted.
- For 100 µL reactions, an intermediate 96-well plate or PCR strips can be used before transferring cells to the final culture vessel.
- 24-well plates can be used for post-electroporation cell seeding with the 8-channel pipette if alternating channels are used for electroporation.

DNA quality and amount

The quality and concentration of DNA used for electroporation plays an important role for the transfection efficiency. We strongly recommend using high quality plasmid purification kits such as PureLink™ HiPure Plasmid DNA Purification Kits (see page 85) to prepare DNA.

- Resuspend the purified DNA in deionized water or TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at a concentration between 1–5 µg/µL. Concentrations may vary depending on cell type.
- The DNA amount should not exceed 10% of total volume used.
- Check the purity of the purified DNA preparation by measurement of the $A_{260/280}$ ratio. The ratio should be at least 1.8 for electroporation.
- The device has been routinely tested with 4–7 kb plasmids and plasmids up to approximately 20 kb should not be a problem. Using plasmids larger than 20 kb will most likely lower transfection efficiency.

IMPORTANT! Do not precipitate DNA with ethanol to concentrate DNA. Concentrated DNA by ethanol precipitation shows poor transfection efficiency and cell viability due to salt contamination.

siRNA quality and amount

The quality and concentration of siRNA used for electroporation plays an important role for the transfection efficiency. We strongly recommend using high quality siRNA such as Stealth™, *Silencer*™ Select, or *Silencer*™ siRNA.

- The recommended starting siRNA concentration is 100–250 µM in nuclease-free water.
- The siRNA amount should not exceed 10% of total volume used.

Controls

GFP control

To evaluate initial transfection efficiency of a cell type by fluorescent microscopy or flow cytometry, using a plasmid encoding GFP (green fluorescent protein) or any colored variant of GFP (Clontech™ or equivalent) is recommended. The plasmid encoding the GFP should have the following features:

- A strong viral promoter (CMV or equivalent) to ensure the highest possible expression level.
- A size of <10 kb.
- High purity with an A_{260}/A_{280} ratio between 1.7 and 1.9. Lower ratios can result in lower transfection efficiency.
- Low endotoxin levels of <10 EU/µg. Use plasmids purified through anion-exchange chromatography to avoid endotoxin contamination.The presence of endotoxins is detrimental to cell health and transfection efficiency.

siRNA control

The success of electroporation is influenced by the type and quality of siRNA used. Therefore, using both a positive and negative control to evaluate the effectiveness of transfecting with siRNA is recommend. Depending on experimental needs, several types of controls are available.

siRNA positive controls	siRNA negative controls
• BLOCK-iT™ Fluorescent Oligo • *Silencer*™ Select GAPDH Positive Control siRNA • Stealth™ RNAi siRNA positive controls • *Silencer*™ positive control siRNAs	• *Silencer*™ Select negative control siRNA • Stealth™ RNAi siRNA negative controls • *Silencer*™ negative control siRNAs

CRISPR/gRNA control

Use TrueGuide™ positive controls (human AVVS1, CDK4, HPRT1, or mouse Rosa 26) and negative control gRNA (non-coding) to determine gRNA amount and transfection conditions that give the optimal gene editing efficiency with the highest cell viability. The TrueGuide™ positive and negative sgRNA and crRNA controls are available separately from Thermo Fisher Scientific. For more information, see **thermofisher.com/trueguide**.

Using the Neon™ NxT Electroporation System

Instructions are provided in this section to use the Neon™ NxT device with the Neon™ NxT Pipette Station and Neon™ NxT Kits for electroporation of mammalian cells.

General instructions to prepare cells for use with the Neon™ NxT Electroporation System are described below. For primary and stem cell types, use the established methods developed in the laboratory.

For videos on how to use the pipette, set up the system, and other information, see **thermofisher.com/us/en/home/life-science/cell-culture/transfection/neon-electroporation-system.html#how-to-video**.

See page 73 if you wish to use a preprogrammed optimization protocol.

Required materials not supplied

See page 85 for ordering information.

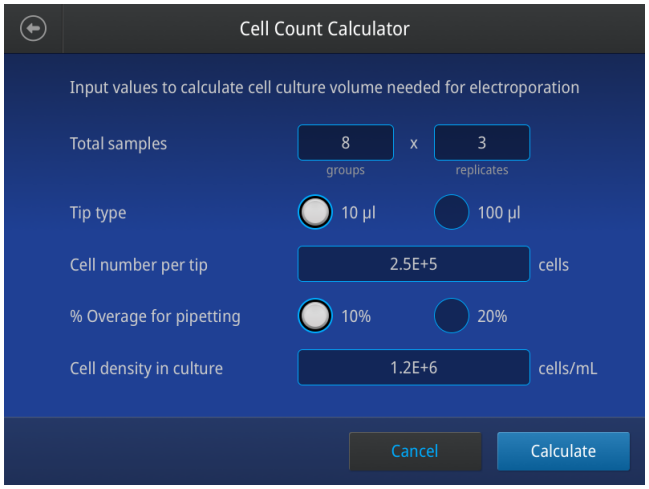
- Cells
- Neon™ NxT Kits
- High quality payload (see page 85 for recommendations)
- Cell culture plates containing the appropriate medium
- D-PBS or Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+}
- Trypsin/EDTA or TrypLE™ Express (Cat. No. 12563) for adherent cells
- Countess™ 3 FL Automated Cell Counter (see page 85)
- Instruments and assays for characterization (see page 85)

IMPORTANT!

- To obtain the highest transfection efficiency, optimize transfection conditions by varying electrical parameters as described in Appendix B, “Optimization” using preprogrammed optimization protocols.
- Since the cell culture conditions vary from user to user, be sure to use low passage number, actively dividing cells (for dividing cells)

Calculate electroporation volumes for cell suspension

Select and enter the appropriate parameters into the calculator to determine the appropriate volumes of cells and buffers to aspirate into the pipette for performing electroporation.



Amount of reagents

For each electroporation sample, the typical amount of payload, cell number, and volume of plating medium per well are listed in the following table.

Format	Cell Type	DNA (µg)	siRNA (nM)	Neon™ NxT Tip	Volume of plating medium	Cell Number	R, T, or Genome Editing Buffer
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
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

Prepare adherent cells

1. Cultivate the required number of cells (70–90% confluent on the day of transfection) by seeding a flask containing fresh growth medium 1–2 days prior to electroporation.

   For most optimized protocols, seed with:

   - 5×10^4 to 2×10^5 cells for each 10 µL Neon™ NxT Tip
   - 5×10^5 to 2×10^6 cells for each 100 µL Neon™ NxT Tip

2. Pre-warm an aliquot of culture medium containing serum, PBS (without Ca^{2+} and Mg^{2+}), and Trypsin/EDTA solution to 37°C.

3. Aspirate the media from cells and rinse the cells using PBS (without Ca^{2+} and Mg^{2+}).

4. Trypsinize the cells using Trypsin/EDTA or TrypLE™ Express (Cat. no. 12563).

5. After neutralization, harvest the cells in growth medium with serum (~0.75 mL for a 10 µL Neon™ NxT Tip or 7.5 mL for a 100 µL Neon™ NxT Tip).

6. Take an aliquot of trypsinized cell suspension and count cells to determine the cell density.

7. Transfer the cells to a 1.5 mL microcentrifuge tube or a 15 mL conical tube and centrifuge the cells at 100–400 × *g* for 5–10 minutes at room temperature.

8. Wash cells with PBS (without Ca^{2+} and Mg^{2+}) by centrifugation at 100–400 × *g* for 5–10 minutes at room temperature.

9. Aspirate the PBS and resuspend the cell pellet in Resuspension R Buffer (or Resuspension T Buffer for programs ≥1900V; or Resuspension Genome Editing Buffer for genome editing applications) at a final density of 1–5 × 10^7 cells/mL. Gently pipette the cells to obtain a single cell suspension.

   Note: Avoid storing the cell suspension for more than 15–30 minutes at room temperature, which reduces cell viability and transfection efficiency. The resuspension cell density may be adjusted to accommodate the recommended cell numbers for the electroporation protocol (see page 50) or optimization protocols (see page 73).

10. Prepare post-electroporation vessels by filling them with pre-warmed culture medium containing serum and supplements **without antibiotics** and preincubate plates in a humidified 37°C/5% CO_2 incubator. If you are using other plate format, see page 51 for plating medium volume recommendations.

Prepare suspension cells

1. Cultivate the required number of cells (cell density ~1–3 × 10^6 cells/T-25 flask) by seeding a flask containing fresh growth medium 1–2 days prior to electroporation.

   For most optimized protocols, seed with:

   - 1–5 × 10^5 cells for each 10 µL Neon™ NxT Tip
   - 1–5 × 10^6 cells for each 100 µL Neon™ NxT Tip

2. Pre-warm an aliquot (500 µL per sample for a 10 µL Neon™ NxT Tip or 5 mL for a 100 µL Neon™ NxT Tip) of culture medium containing serum. Also prepare an appropriate volume of PBS (without Ca^{2+} and Mg^{2+}).

3. Take an aliquot of cell culture and count the cells to determine the cell density.

4. Transfer the cells to a microcentrifuge tube or 15 mL conical tube and pellet the cells by centrifugation at 100–400 × *g* for 5–10 minutes at room temperature.

5. Wash the cells with PBS (without Ca^{2+} and Mg^{2+}) and pellet the cells by centrifugation at 100–400 × *g* for 5 minutes at room temperature.

6. Aspirate the PBS and resuspend the cell pellet in Resuspension R Buffer (or Resuspension T Buffer for programs ≥1900 V; or Resuspension Genome Editing Buffer for genome editing applications) at a final density of 1–5 × 10^7 cells/mL. Gently pipette the cells to obtain a single cell suspension.

   Note: Avoid storing the cell suspension for more than 15–30 minutes at room temperature, which reduces cell viability and transfection efficiency. The resuspension cell density maybe adjusted to accommodate the recommended cell numbers for the electroporation protocol (see page 50) or optimization protocols (see page 73).

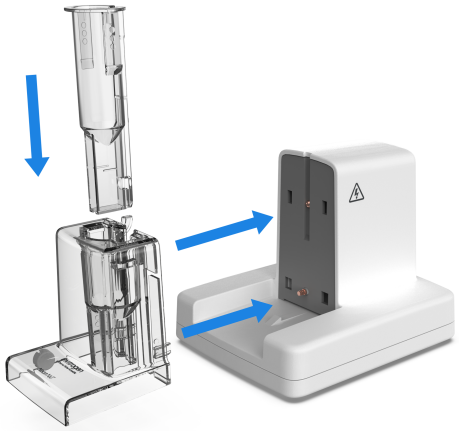
7. Prepare post-electroporation vessels by filling them with pre-warmed culture medium containing serum and supplements **without antibiotics** and preincubate plates in a humidified 37°C/5% CO_2 incubator. If you are using other plate formats, see page 51 for plating medium volume recommendations.

Set up the pipette station

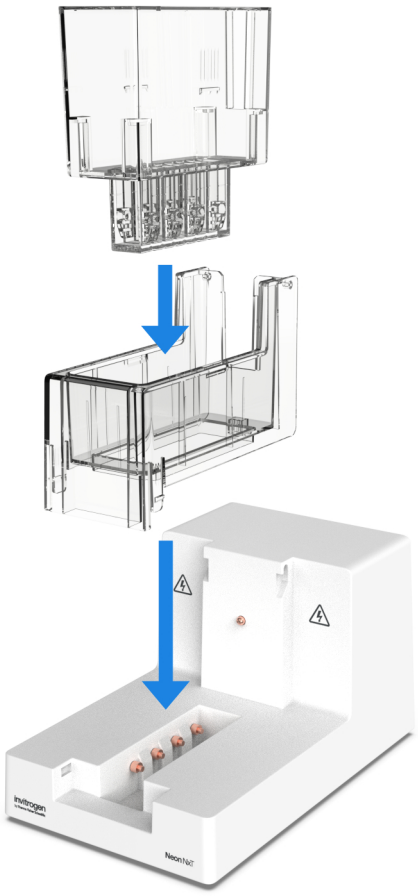
1. Ensure the Neon™ NxT Pipette Station is connected to the Neon™ NxT device (see 20).
2. (*If necessary*) Attach the Tube Chamber for Neon™ NxT Tubes to the Neon™ NxT Pipette Station.
3. Fill each chamber of the Neon™ NxT Tube with 2 mL of electrolytic buffer **(use Buffer E10 for 10 µL Neon™ NxT Tips and Buffer E100 for 100 µL Neon™ NxT Tips)**.

   Note: The liquid level in the electroporation tube should reach the level of the indicator line. Make sure that the electrode on the side of the tube is completely immersed in buffer.

4. Insert the Neon™ NxT Tube into the tube chamber, until you hear a click as the tube snaps in place.



1-Channel setup



8-Channel setup

5. The station is ready for use. When ready, proceed to “Load the pipette” on page 55.

Load the pipette

For details on how to handle Neon™ NxT pipettes, see “Using the Neon™ NxT 1-Channel Pipette” on page 14 or “Using the Neon™ NxT 8-Channel Pipette” on page 16.

1. Open the box containing the type of Neon™ NxT Tip to be used for electroporation.

   Note: As few tips as wanted can be used with the 8-channel pipette (including using four tips and alternating them to use with a 24-well plate). However, it is recommended that if using less than four tips, to switch to the 1-channel pipette. This will provide a better electroporation experience, conserve 8-channel tubes, and prevent cross-contamination.

2. Place the end of the Neon™ NxT Pipette into the Neon™ NxT Tip, and apply gentle downward pressure until the tip snaps into place.

3. Press the plunger on the Neon™ NxT Pipette all the way down to allow the tip holder to grip the piston in the tip.

   The Neon™ NxT Tip should be tightly connected to the tip holder with no gaps to ensure trouble-free pipetting and proper electrical connection.

4. Transfer the appropriate amount of payload into a sterile vessel.
   - Use a 1.5 mL microcentrifuge tube for a 1-Channel setup.
   - Use a loading trough for an 8-Channel setup.

5. Add cells to the vessel containing the payload and gently mix.

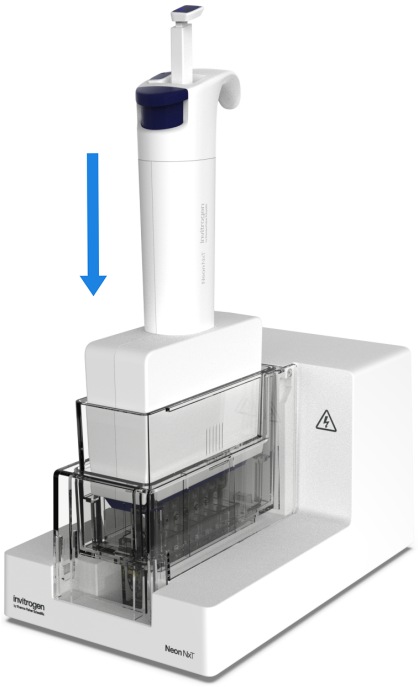
6. Press the plunger on the Neon™ NxT Pipette to the first stop and immerse the tip into the cell-payload mixture. Slowly release the plunger to aspirate the cell-payload mixture into the Neon™ NxT Tip.

   Note: Ensure that the piston is fully retracted before removing the tip from the mixture to avoid air bubbles.

   Air bubbles cause arcing during electroporation leading to lowered or failed transfection. If there are air bubbles in the tip, discard the sample and carefully aspirate a fresh sample without air bubbles into the tip.

7. Dock the Neon™ NxT Pipette with the sample vertically into the Neon™ NxT Tube placed in the Neon™ NxT Pipette Station until it clicks into place. Ensure that the pipette is securely docked in the pipette station, and that the tips are submerged in electrolytic buffer.

1-Channel setup



8-Channel setup

8. The station is ready for use. Proceed to “Run electroporation protocol” on page 57.

Run electroporation protocol

1. Ensure that the recommended amount of payload, cell number, and volume of plating medium is used for each electroporation sample.

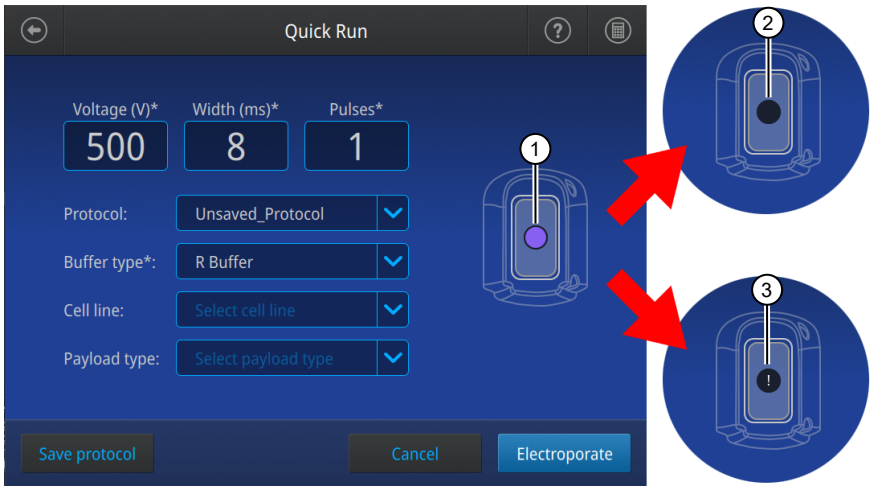
2. Select the protocol or plate map that you want to use to perform electroporation.
   a. Select **Set up run** ▸ **Open plate** to select an existing plate map.

   b. Select **Set up run** ▸ **Create plate** to create a plate map.

   c. Select **Set up run** ▸ **Quick run** to select or create a protocol.

3. Select **Electroporate** to start electroporation.

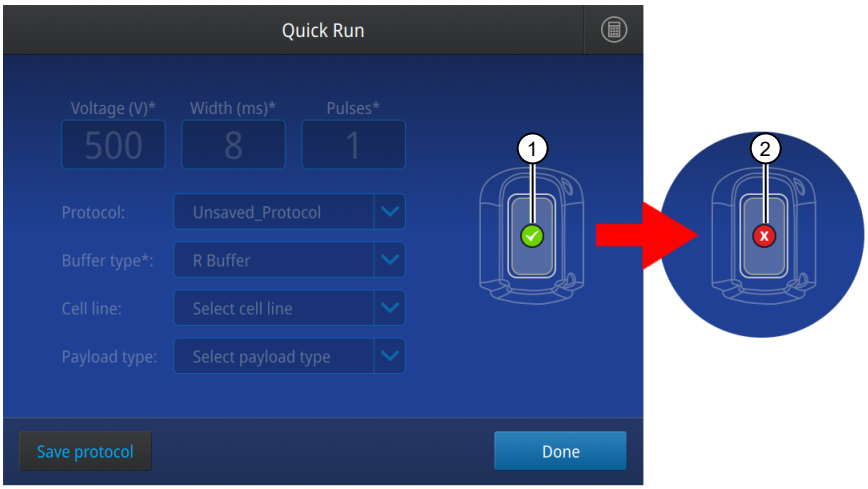
4. The Neon™ NxT device checks for the proper insertion of the Neon™ NxT Tube and Neon™ NxT Pipette before delivering the electric pulse.



① Tip detected, proceed with protocol
② No pipette detected
③ Tip detected, but error (select the circle to view the error message)

Note: Connection errors and the presence of bubbles in the tip are two of the most common errors encountered (see Appendix A, “Troubleshooting”). Bubbles can cause arcing (sparks) during electroporation and results in low transfection efficiency and cell viability.

5. After delivering the electric pulse, **Complete** is displayed on the main dial to indicate that electroporation is complete.



① Protocol completed normally
② Protocol failed (select the circle to view the error message)

6. Slowly remove the Neon™ NxT Pipette from the Neon™ NxT Pipette Station and immediately transfer the samples from the Neon™ NxT Tip by pressing the plunger on the pipette to the first stop into the prepared culture plate containing prewarmed medium.

Note: Load electroporated cells into growth medium **without antibiotics** to avoid reduction of cell viability after transfection.

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7. To discard the Neon™ NxT Tip, press the eject button to disengage the tip, then press the plunger to the second stop to eject the tip.

   See "Using the Neon™ NxT 1-Channel Pipette" on page 14 or "Using the Neon™ NxT 8-Channel Pipette" on page 16 for details.

Note: Ensure the tip is discarded into an appropriate biological hazardous waste container.

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8. Repeat the procedure described in "Load the pipette" and "Run electroporation protocol" for the remaining samples.

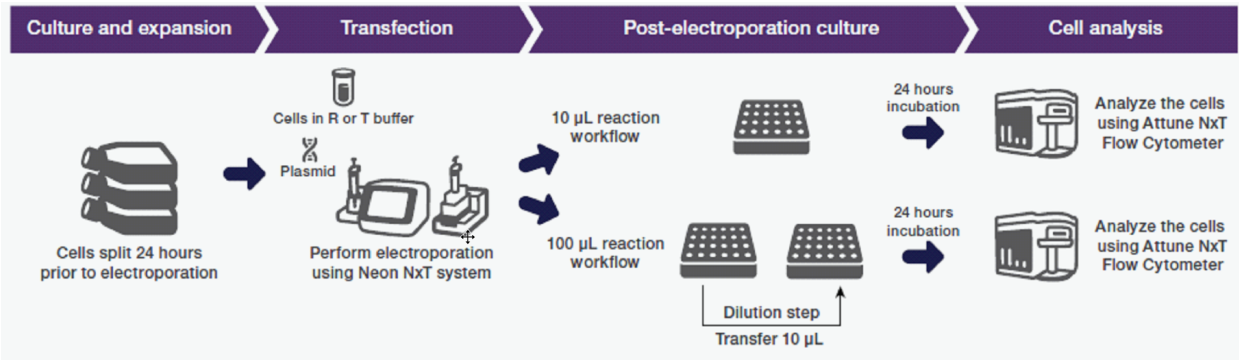
   Change the Neon™ NxT Tips after two uses and Neon™ NxT Tubes after 12 uses. Use a new Neon™ NxT Tip and Neon™ NxT Tube for each new payload.

9. If you are not using the Neon™ NxT device, turn the power switch on the rear to **OFF**.

Post-electroporation process

After electroporation, allow the electroporated cells to recover by incubating at 37°C for 24 hours.

For 100 µL reactions, an intermediate 96-well plate or PCR strips can be used before transferring cells to the final culture vessel. For an example in the following workflow, a 96 well plate was used as an intermediate plate. Following this step, 10 µL of the electroporated sample from the intermediate plate is transferred into a 96-well plate containing 200 µL of media in each well. After transferring the samples, incubate the plate containing both the media and the electroporated samples for 24 hours.



After the cells have recovered, assay samples to determine the transfection efficiency (e.g., by flow cytometry), or for gene knockdown or knockout efficiency.

Seed electroporated cells

1. Plate the cells in the preincubated vessels containing culture media containing serum and supplements without antibiotics described in “Prepare adherent cells” (step 10) or “Prepare suspension cells” (step 7).
2. Gently rock the plate to assure even distribution of the cells.
3. Incubate the plate at 37°C in a humidified CO_2 incubator for 24 hours.

Maintenance

Cleaning and maintenance

CAUTION! Cleaning and decontamination. Use only the cleaning and decontamination methods specified in the user documentation. It is the responsibility of the operator (or other responsible person) to ensure the following requirements are met:

- No decontamination or cleaning agents are used that can cause a HAZARD as a result of a reaction with parts of the equipment or with material contained in the equipment.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) prior to having the instrument serviced at your facility or sending the instrument for repair, maintenance, trade-in, disposal, or termination of a loan (decontamination forms may be required from customer service).
- Before using any cleaning or decontamination methods (except those recommended by the manufacturer), users should confirm with the manufacturer that the proposed method will not damage the equipment.

Clean the surface of the Neon™ NxT Electroporation System with a damp cloth. **Do not** use harsh detergents or organic solvents to clean the unit.

The instrument (chassis, electrodes and screen) shall be resistant to the following chemicals:

- Cleaning agents containing 70% ethanol, 70% isopropanol, 0.6% sodium hypochlorite.
- Cleaning solutions containing DMSO.

In case liquids (e.g., buffer, water, coffee) are accidentally spilled inside the instrument, wipe the spill using dry laboratory paper.

For any other repairs and service, contact Technical Support. **Do not** perform any repairs or service by yourself to avoid damage to the instrument or voiding the warranty.

Cleaning and maintenance

Clean the surface of the Neon™ NxT device and Neon™ NxT Pipette Station with a damp cloth. **Do not** use harsh detergents or organic solvents to clean the unit. The Neon™ NxT Pipette is permanently calibrated at the manufacturer and does not require any further calibration.

IMPORTANT! Avoid spilling any liquid inside of the Neon™ NxT Pipette Station to prevent any build up of rust on the ball plunger in the pipette station.

In case you accidentally spill any liquid (e.g., buffer, water, coffee) inside the Neon™ NxT Pipette Station, disconnect the station from the main device and wipe the station using dry laboratory paper. Invert and allow the station to completely dry for 24 hours at room temperature. **Do not use the oven to dry the Neon™ NxT Pipette Station.** If the station does not work after drying, contact Technical Support.

For any other repairs and service, contact Technical Support. **Do not** perform any repairs or service on the Neon™ NxT device yourself as it is a high voltage hazard and to avoid any damage to the unit or voiding your warranty.

Replace the fuses

Required materials

- Two IEC/UL listed fuses, rated 10 A, type T (time-lag), 250 VAC, 5 × 20 mm
- Small flat-tip screwdriver

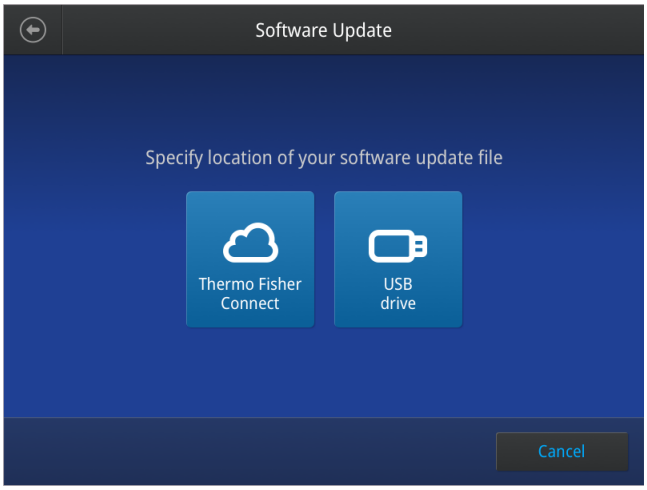
Replace fuses

DANGER! ELECTRICAL SHOCK HAZARD. Severe electrical shock, which could cause physical injury or death, can result from working on an instrument when the high voltage power supply is operating. To avoid electrical shock, disconnect the power supply to the instrument, unplug the power cord, and wait at least 30 minutes to allow the high voltage capacitor to be fully discharged before working on the instrument.

1. Power off the instrument, then disconnect the power cord from the instrument.
2. Use a flat-tip screwdriver to remove the fuse compartment from the instrument.
3. Remove the fuse from the fuse compartment for inspection.
4. Replace blown fuse(s) with new IEC/UL listed fuses, rated 10 A, type T (time-lag), 250 VAC, 5 × 20 mm.
5. Replace the fuse compartment in the instrument.
6. Reconnect the instrument power cord.

Upgrade the system firmware

Update software directly through Connect or using an USB drive with updated software downloaded from **thermofisher.com/connect**.



Determine firmware version on instrument

When a new firmware version is released, you may be required to load the new firmware on the instrument.

You will need a USB memory device and, if your instrument requires login, the login details to upgrade the firmware.

1. Select **(Settings)** > **About Instrument**.
2. View current firmware version.

Upgrade the instrument firmware (Cloud)

IMPORTANT! You cannot upgrade the firmware while a run is in progress.

1. Select **(Settings)** ▸ **Maintenance & Services** ▸ **Software Update** ▸ **ThermoFisher Connect**.
2. Select **Yes** to start the upgrade.

   IMPORTANT! To prevent instrument malfunction and required service, do not power off the instrument during the upgrade.

   When the upgrade process is complete, the instrument will automatically restart.

Download new firmware

1. Go to **thermofisher.com** from your web browser.
2. Click **Product Support** ▸ **Technical Resources** ▸ **Product Support** ▸ **Software, Patches & Updates**.
3. Select Neon™ NxT Electroporation System in the list, then click **Updates & Patches**.
4. Find the appropriate file. If the version number is:
   - The same as the current version on the instrument, you do not need to upgrade the firmware.
   - Different from the current version on the instrument, download the new firmware.
5. Insert a USB memory device into the USB port on the computer.
6. Click the link in the Software column, then select the USB memory device as the location for the saved file.

   Note: The file must be downloaded to the root directory of the USB memory device and not into a folder.
7. Remove the USB memory device from the computer when the download is complete.

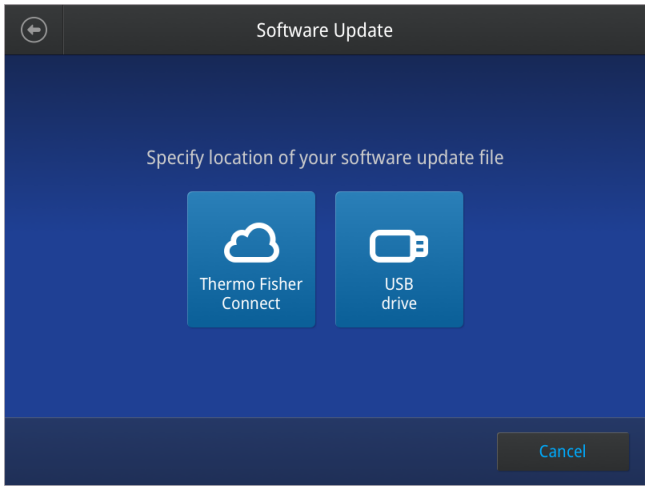
Upgrade the instrument firmware (USB drive)

IMPORTANT! You cannot upgrade the firmware while a run is in progress.

1. Insert the USB memory device (FAT32 format file system) with the new firmware in the USB port of your instrument.

   Note: For instruments with the USB shortcuts feature enabled, you will be directed to the **USB shortcuts** screen. Select **Update Software** to proceed to the **Software Update** screen.

2. Select **(Settings)** ▸ **Maintenance & Services** ▸ **Software Update** ▸ **USB drive**.
   The Software Update screen opens:



3. Choose the row with the new firmware file from the USB memory device, then **Select**.

4. Select **Yes** to start the upgrade.

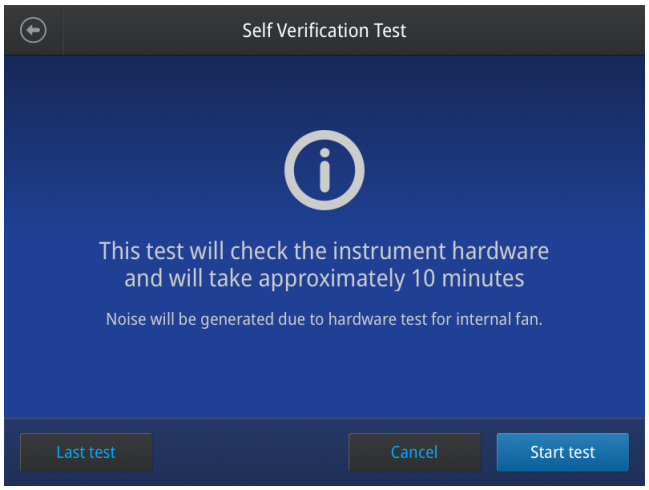
   IMPORTANT! To prevent instrument malfunction and required service, do not power off the instrument during the upgrade.

   When the upgrade process is complete, the instrument will automatically restart.

Self Verification Test

Use the **Self Verification Test** feature to check the instrument hardware. The check includes testing the high voltage system and other components.

Carry out the **Self Verification Test** periodically or whenever there is an intermittent instrument error. Contact your service representative in case of block failure.

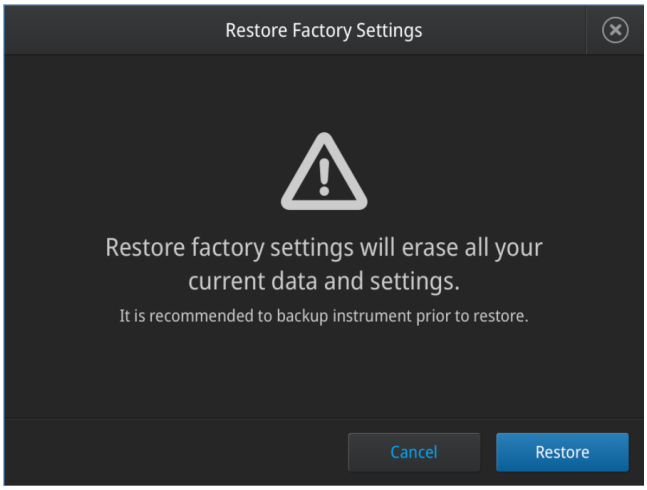


Perform self verification test

1. Connect a Neon™ NxT Pipette Station and insert an empty Neon™ NxT Tube, then and dock a Neon™ NxT Pipette WITHOUT a Neon™ NxT Tip.
2. (*Optional*) Select **Last test** to view the results of the last **Self Verification Test**.
3. Select **Start test** to test the device.
4. Remove the Neon™ NxT Pipette from the Neon™ NxT Pipette Station and attach a Neon™ NxT Tip.
5. Add E10 or E100 buffer to the Neon™ NxT Tube and aspirate E10 or E100 buffer with the Neon™ NxT Pipette to fill the tip.
6. Dock the Neon™ NxT Pipette in the Neon™ NxT Tube.
7. Repeat the process for the Neon™ NxT 8-Channel Pipette Station as directed on the screen.

Restore factory settings (*Administrator profile only*)

Select **Restore factory settings** to remove all the data and customized settings and revert to factory settings. All data and settings will be erased once factory settings are restored. At the end of the restoration process, the message, "Your instrument has been restored." is displayed and the instrument automatically reboots after 30 seconds.



Repackaging the instrument

If you need to send the instrument to Thermo Fisher Scientific for warranty issues, or you wish to transport the instrument to another location, repackage the unit as follows.

Note: Prior to sending the instrument, ensure the instrument is properly decontaminated if the instrument is exposed to any viable biological agents, radioactive materials, or hazardous chemicals (toxic, carcinogenic, mutagenic, toxic for reproduction, sensitizing, and/or have not been fully tested). Contact Technical Support for a decontamination protocol and to obtain a Returns Goods Authorization (RGA) number and return shipping instructions.

Repackaging and storage instructions

1. Turn off the main power switch at the rear of the instrument and detach the power cord from the rear of instrument.
2. Place the instrument in the original box including the original packing foam.
3. Tape the box securely and place appropriate shipping labels for shipping the instrument to Thermo Fisher Scientific. Always transport the box with the unit in the **upright** position.
4. If the instrument is not to be used for extended periods of time, store the repackaged instrument in an upright position at 4°C to 40°C.

A

Troubleshooting

Observation	Possible cause	Recommended action
No power (the display remains blank when the power is turned on)	AC power cord is not connected.	Check AC power cord connections at both ends. Use the correct cords.
Cannot detect pipette	The pipette is not fully inserted into the tube.	Ensure the Neon™ NxT Tube is inserted properly in the Neon™ NxT Pipette Station.
		Ensure the Neon™ NxT Pipette is inserted properly in the Neon™ NxT Tube.
Cannot detect station	The Low voltage interface connector is not connected.	Ensure the low voltage interface connector of the Neon™ NxT Pipette Station is properly connected to the low voltage interface port at the rear of the Neon™ NxT device.
		Check the low voltage interface connector on both pulse generator and pipette station. Make sure the marks on the cable plug and instrument connector are aligned correctly.
Cannot detect transparent safety cover	Safety cover is not properly installed.	Check the safety cover and make sure it is properly installed.
Error message	Various.	See "Instrument error codes" on page 68 for a description of error messages.
Connection failure	Pipette or tube is incorrectly inserted.	Ensure the Neon™ NxT Pipette is inserted properly in the Neon™ NxT Pipette Station (see "Run electroporation protocol" on page 57.
		Ensure the Neon™ NxT Tube is inserted properly in the Neon™ NxT Pipette Station (see "Run electroporation protocol" on page 57.
	Bubbles present in the Neon™ NxT Tip.	Reload the tip, making sure that the piston is fully retracted before removing it from cell-DNA/siRNA mixture.
	No Neon™ NxT Tip is inserted or the Neon™ NxT Tip is inserted incorrectly.	Make sure the Neon™ NxT Tip is correctly inserted in the Neon™ NxT Pipette. There should be no gap between the tip and the top head of the pipette.
	No buffer in the tube or no sample in the tip.	Be sure to add 2 mL of the appropriate electrolytic buffer to the Neon™ NxT Tube. The electrode in the tube must be completely immersed in buffer.
		Ensure that samples in Resuspension Buffer are loaded into the Neon™ NxT Tip.

Observation	Possible cause	Recommended action
Connection failure *(continued)*	Wrong buffers used.	Use electrolytic buffer (Buffer E for 10 µL tip and Buffer E2 for 100 µL tip) in the Neon™ NxT Tube and sample in Resuspension Buffer in the Neon™ NxT Tip. Do not switch buffers or use any other buffer as these buffers are specifically designed for electroporation with the Neon™ NxT device.
	High voltage connector is not connected.	Be sure to connect the high voltage connector of the Neon™ NxT Pipette Station to the high voltage port on the rear of the Neon™ NxT device.
Persistent connection failure	Perform self diagnostics test.	Perform self diagnostics test by **Maintenance & Services ▸ Self verification test**. During the self diagnostics test, the device checks a variety of parameters and indicates if it is OK or there is a problem. If the self diagnostics is OK, ensure that all connections are correct as described in this section before contacting Technical Support.
Arcing (sparks)	Air bubbles in the Neon™ NxT Tip.	Avoid any air bubbles in the Neon™ NxT Tip while aspirating the sample.
	High voltage or pulse length settings.	Reduce the voltage or pulse length settings.
	Accidentally used salt-precipitated DNA.	Do not precipitate DNA with ethanol to concentrate DNA as it can cause arcing due to salt contamination.
Low cell survival rate	Poor DNA quality.	Use high quality plasmid DNA for transfection (see page 48 for guidelines and recommendations on DNA quality).
	Cells are stressed or damaged.	Avoid severe conditions during cell harvesting especially high speed centrifugation and pipette cells gently.
		Avoid using over confluent cells or cells at high densities as this may affect the cell survival after electroporation.
		After electroporation, immediately plate the cells into prewarmed culture medium **without antibiotics**.
	Multiple use of the same Neon™ NxT Tip.	**Do not** use the same Neon™ NxT Tip for electroporation more than twice. Repeated application of electric pulses reduces the tip quality and impairs their physical integrity.
Low transfection efficiency	Poor optimization of electrical parameter.	Perform optimization for your cell type as described on page 73.
	Poor plasmid DNA quality or the plasmid DNA is low.	Use high quality plasmid DNA for transfection (see page 48 for guidelines and recommendations on DNA quality).
		Start with 0.5 µg plasmid DNA per sample.
	Incorrect cell density.	Cell densities >3×10^5 or <5×10^4 per sample drastically reduces transfection efficiency. Use 5×10^4–1.5×10^5 cells per 10 µL per sample.
	Mycoplasma contaminated cells.	Test cells for *Mycoplasma* contamination.
		Start a new culture from a fresh stock.
Irreproducible transfection efficiency	Inconsistent cell confluency or passage number.	Always use cells with low passage number and harvest cells with comparable confluency levels.

Observation	Possible cause	Recommended action
Irreproducible transfection efficiency *(continued)*	Multiple use of Neon™ NxT Tip and Neon™ NxT Tube.	**Do not** use the same Neon™ NxT Tip more than **twice**. Repeated application of electric pulses reduces the tip quality and impairs their physical integrity.
		Do not use the same Neon™ NxT Tube for more than 12 times.
		Always use a new Neon™ NxT Tip and Neon™ NxT Tube for electroporation of different plasmid DNA samples to avoid cross-contamination.
High energy error	Used high electrical parameters.	Set lower voltage or duration.

Instrument error codes

This section describes possible error messages displayed by the instrument. Most of the error messages are self explanatory and after fixing the error, you should be able to continue with the protocol. Contact Technical Support if it is necessary to send the device for service.

Error message	Possible cause	Action
Protocol setup		
Total energy above arc limit	Electroporation parameters input above arc limit	The protocol risk arcing during electroporation. Confirm to create the protocol. Cancel to change parameters value.
Total energy above hardware limit	Electroporation parameters input above hardware limit	Lower one or more of the electroporation parameters.
Save protocol or Save plate		
Protocol/plate template cannot be overwritten	Save protocol/plate same name as template protocols	Edit the name or save the file with a different name.
Other user's protocol/plate cannot be overwritten	Save protocol/plate same name as other user's protocol	Edit the name or save the file with a different name.
Protocol with same name exists	Save protocol same name as own existing protocol	Save as new file with a different name.
Plate with same name exists	Save plate same name as own existing plate	Save as new file with different name or click overwrite to overwrite the existing plate.
Please enter protocol/plate name	Save protocol/plate without name	Enter the protocol/plate name to save the protocol.
Max number of saved protocols/plates reached	Save protocol/plate when max numbers of protocol/plate is reached	Remove some protocols/plates and try to save again.

(continued)

Error message	Possible cause	Action
Mange protocol or Mange plate		
Selected protocols/plates cannot be deleted	Delete default protocols	Check selected protocols/plates and try again.
Quick run setup		
Connect pipette station and ensure transparent safety guard is installed to start	Pipette station or transparent guard is not connected or install	Connect the pipette station and ensure the transparent safety guard is installed.
Tip connection error	Tip detected not valid to run electroporation (eg. Bubbles or empty tip)	Check tip is attached properly. Ensure attached tip does not contains air bubbles. Select the **?** button and follow the steps for loading consumable details if needed.
Plate run setup		
Connect pipette station to start	Pipette station not connected	Connect the pipette station.
Ensure the transparent safety guard is installed to start	Transparent safety guard is not installed	Ensure the transparent safety guard is installed.
Dock the pipette to electroporate	Pipette is not docked	Dock the pipette to electroporate.
Tip connection error	Tip detected not valid to run electroporation (e.g., bubbles or empty tip)	Check tip is attached properly. Ensure attached tip does not contains air bubbles. Select the **?** button and follow the steps for loading consumable details if needed.
Run protocol		
Tip type is unknown	Tip detection error during electroporation run	Check tip is attached properly. Ensure attached tip does not contain air bubbles.
Please make sure all components are connected properly and try again	Components are not connected when electroporation is initiated but not started	Check all components (pipette station, transparent guard, and pipette) are connected and attached properly.
Station not detected	Station removed/not detect during run	Attach pipette station and do not remove it during run.
Pipette removed during electroporation. Run aborted	Pipette removed/not detected during run	Attach pipette and do not remove it during run.
Arcing detected during electroporation	Arcing detected during run	Parameters input cause arcing. Lower the electroporation parameters for the next run if needed.

(continued)

Error message	Possible cause	Action
High voltage safety fault	Discharging failed, unable to discharge within time before the next run	Do not power off instrument or touch/remove the pipette until voltage is down to safe level/allowed to continue.
Profile errors		
Invalid PIN. PIN in sequential order or repetitive numbers are not allowed	Invalid PIN during creating new profile	Enter PIN that is not in sequential order (e.g., 1234, 5678) or not repetitive numbers (eg. 1111, 0000).
Invalid usernamel	Empty or invalid username	Please input 1–20 alphanumeric characters and no spaces for the username.
Cloud connection errors		
No internet connection	Link account to cloud without internet connection	Ensure the instrument's network is configured and connected to the internet before linking to cloud.
Unable to link to cloud	Unable to link account to cloud after select method of connect (QR/link)	Ensure the instrument's timezone, date, and time is set properly. Reboot once it is done.
USB drive errors		
No USB drive detected	USB drive is not connected or inserted before operations (eg. Import/export files, software update)	Make sure USB drive is inserted and connected properly.
Network drive errors		
Failed to connect to network drive	Unable to connect network drive	Ensure network details are correct and try again.
Instrument settings errors		
Network is not connected	Network cable is not connected when setting up network using wired connection	Check network cable is connected and try again.
USB Wifi card not detected	USB wifi card is not connected when setting up wireless connection	Check USB Wifi card is inserted and connected properly.
Invalid entries/Failed to connect	Invalid entries while setting up network	Ensure the network details are input properly.
Fatal errors (indicated by amber LED)		

(continued)

Error message	Possible cause	Action
Unable to recover from upgrade failure. – 0x8867	Fatal error	Reboot instrument, run a self-test. If the error is persistent contact service representative.
Unknown initialization error. – 0x8855		
Power supply voltage exceeded upper or lower limits. - 0x8300		
Unable to initialize subsystems at initialization. – 0x8854		
NVRAM key not found. – 0x8021		
Fault encountered during charging. Charger over temperature. – 0x814C		
Fault encountered during charging. Charger capacitor over voltage. – 0x814E		
Safety relay turn off failure. – 0x8163		
LV ON Relay failure. – 0x816F		
Power On Test Failure. – 0x8856		
Unable to power up instrument. – 0x8857		
Unable to save more data, instrument filesystem is full. – 0x8870		
Unable to read upgrade package version. – 0x8865		
Unable to locate upgrade package. – 0x8864		
Unable to extract upgrade files. – 0x8863		
Unable to verify upgrade package signature. – 0x8862		
Fan is faulty – 0x8302		

(continued)

Error message	Possible cause	Action
Discharge resistor is faulty – 0x8303	Fatal error	Reboot instrument, run a self-test. If the error is persistent contact service representative.
High Voltage CAP charger is faulty – 0x8301		

B

Optimization

Optimization protocol

Electroporation is a process involves applying electrical energy to cells to achieve temporary pores that allow for the transfer of genetic material across the cell membrane. The process is dependent on the combination of electric parameters such as voltage, pulse width, pulse interval, and pulse number.

- The voltage is the strength or intensity of the electrical energy and is the most critical factor influencing transfection efficiency and viability.
- Pulse width, or duration of how long the voltage is applied to the cell, correlates with the size of the pore created in the cell membrane.
- Pulse number, or multiple pulses can be used to create more pores on the membrane as well as drive charged payloads (DNA or RNA) into the cell cytosol.
- Pulse interval is the distance (or time) between pulses in a multi-pulse profile.

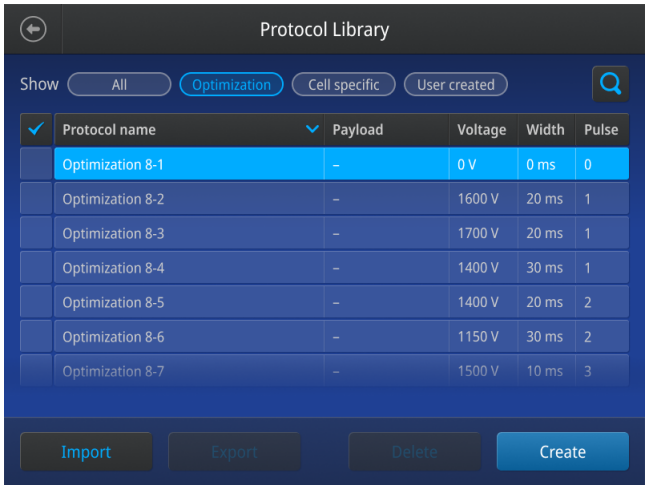
Based on initial results, it may be necessary to optimize the electroporation parameters for a particular cell type and payload. If properly optimized to overcome the transmembrane potential of the cellular lipid bilayer, the delivery efficiency of electroporation can ensure successful genetic manipulation of various cell types for many applications.

The Neon™ NxT Electroporation System provides the ability to program a wide range of combinations for voltage, pulse width, and pulse number. These parameters can be set within the following integer ranges:.

- Voltage: 500 to 2500 volts
- Pulse width: 1 to 100 milliseconds (ms)
- Pulse number: 1 to 10 pulses
- Pulse interval: 1 second (fixed)

The Neon™ NxT Electroporation System is preprogrammed with 24 optimization protocols that have been found to provide a successful starting point for electroporation parameter optimization.

Perform optimization using the Neon™ NxT Electroporation System by preparing cells for each of the 24 protocols (or streamline the process by selecting 8 protocols), then use the protocol that gives the best result for subsequent experiments, or for further optimization.



Optimization of higher voltage programs

A notable advantage of the Neon™ NxT 8-channel system is its flexibility in applying different programs to different wells. This feature simplifies the optimization of multiple programs for specific applications with minimal effort. Users have the freedom to choose any combination of these parameters.

However, it is important to ensure that the total electrical energy for the pulse discharge remains within a certain limit. Each resuspension buffer type has a specific limit for this total energy. If the total energy exceeds the limit of the resuspension buffer, the instrument will display an error message.

Buffers with lower conductivity, such as T buffer, are compatible with higher voltage and energy programs. However, it is important to note that higher energy protocols can lead to lower cell viability.

To reduce the total electrical energy while using higher voltage programs, lower pulse numbers and pulse widths can be employed. This approach can result in significant improvement in electroporation performance for certain application.

For reference, the following table lists recommended higher voltage programs with lower pulse numbers and pulse widths:

Table 5 Higher voltage protocols

Protocol No.	Voltage	Pulse width	Pulse number
P25	1800	10	1
P26	1900	10	1
P27	2000	10	1
P28	2100	10	1
P29	2200	10	1
P30	2300	10	1
P31	2400	10	1
P32	2500	10	1

Table 5 Higher voltage protocols *(continued)*

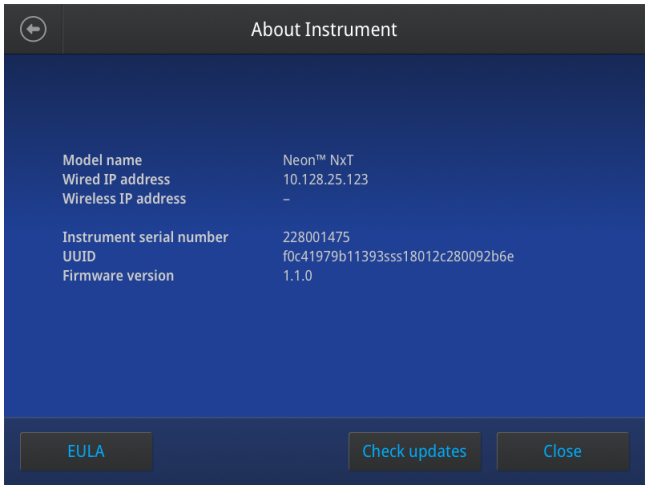
Protocol No.	Voltage	Pulse width	Pulse number
P33	2000	5	2
P34	2100	5	2
P35	2200	5	2
P36	2300	5	2
P37	2400	5	2
P38	2500	5	2
P39	2000	5	3
P40	2100	5	3
P41	2200	5	3
P42	2300	5	3
P43	2400	5	3
P44	2500	5	3
P45	1800	15	1
P46	1900	15	1
P47	2000	15	1
P48	2100	15	1

C Recommended instrument settings

About Instrument

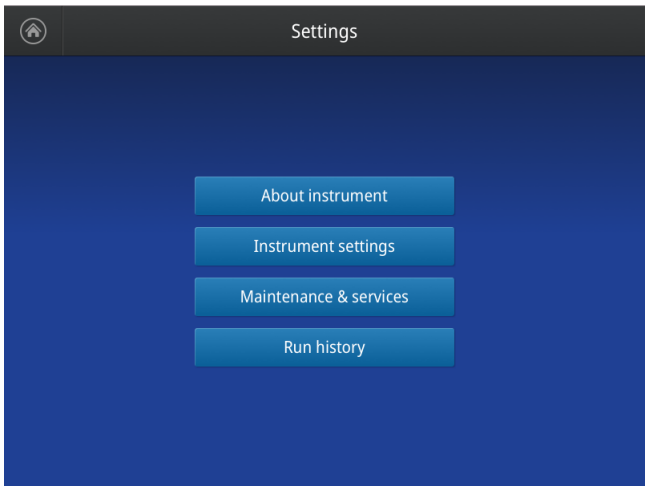
Select **Settings** ▸ **About Instrument** to find out more information about the instrument (e.g., firmware version and instrument statistics).

- Select **EULA** to view the End User License Agreement, or download it to a USB drive.
- Select **Check updates** to find out if updates are available for the instrument .



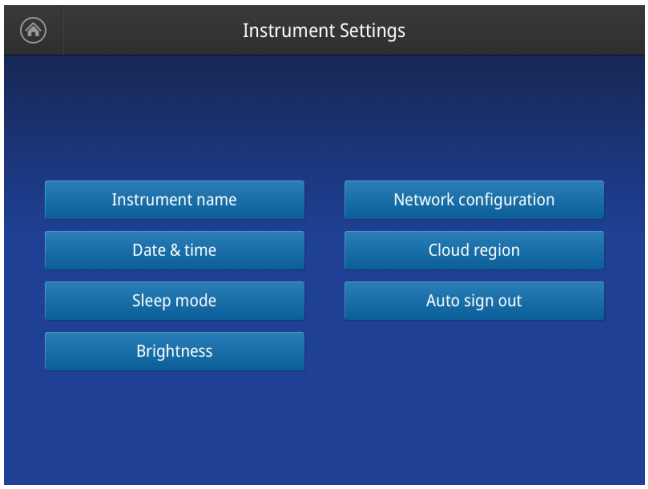
Recommended instrument settings

Select **(Settings)** and access the **Settings** screen to configure the instrument.



Instrument settings (Administrator only)

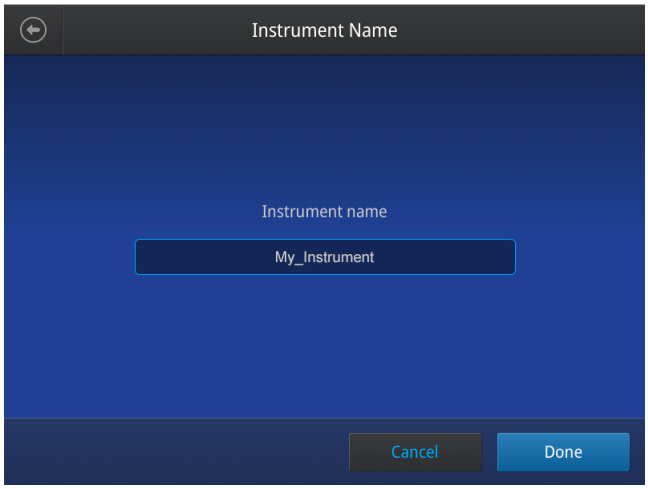
Select **Instrument settings** to set the following instrument parameters.



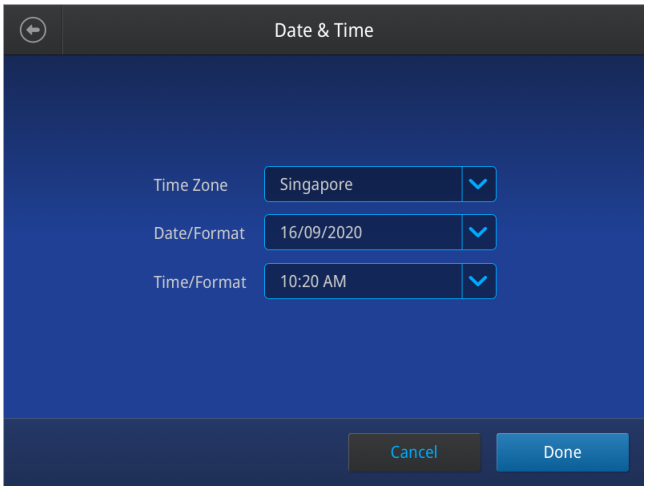
- **Instrument name**

  Select the **Instrument name** field to activate the text editor. Enter up to 25 alphanumeric characters to identify the instrument.

  Note: The instrument name cannot have spaces. Separate consecutive characters with a hyphen or underscore; for example, *My_Instrument*.

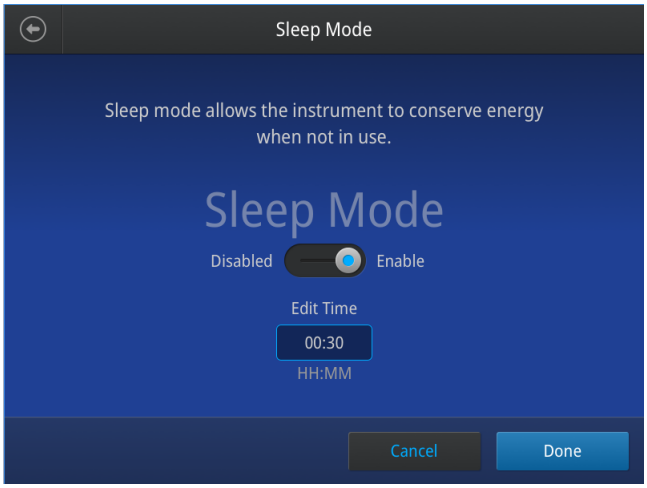


- **Date and time**
  - Select the **Time Zone** field to set the time zone.
  - Select the **Date/Format** field to choose the date format and set the date.
  - Select the **Time/Format** field to activate the numeric editor to set the time.

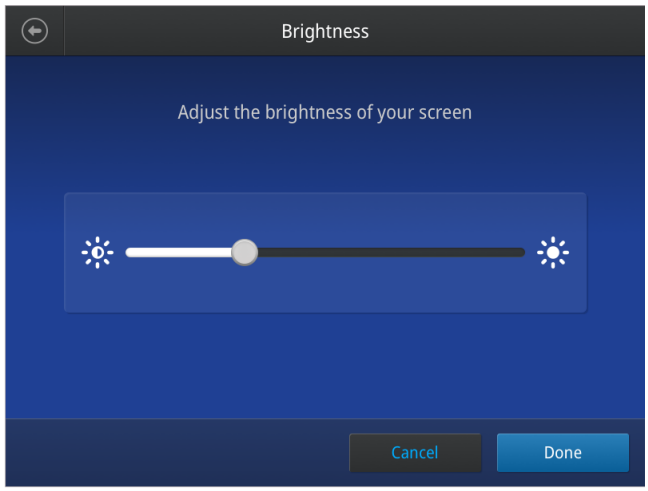


- **Sleep mode**

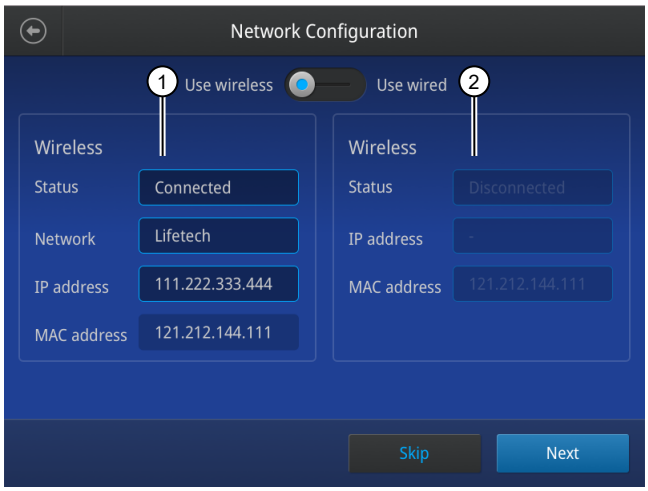
  Use the **Off** and **On** toggle to disable or enable sleep mode. In the 'On' mode, select the **Edit Time** field to activate the numeric editor to set the time after which the instrument will go from idle mode to standby mode.



- **Brightness**
  Use the slider to adjust the brightness of the touch screen.



- **Network configuration**
  Use the toggle to select the type of network connection to connect the instrument to the Internet . For details on using the Wireless and Ethernet options, see “Connect the instrument to the Internet” on page 27.

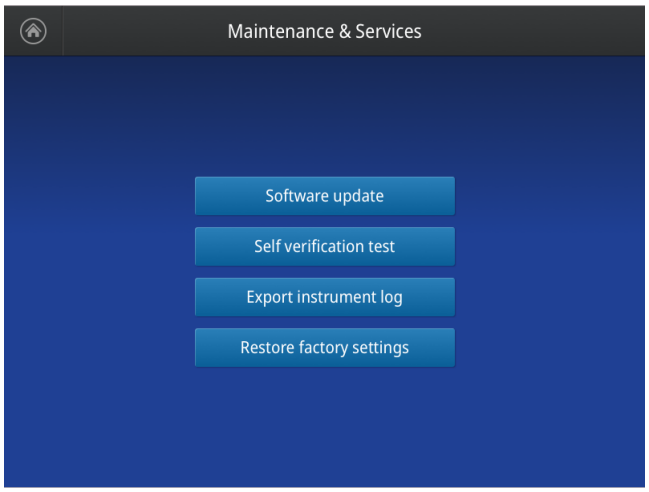


① Wireless panel
② Wired panel

- **Cloud region (*Administrator profile only*)**
  Select the appropriate field to set the cloud region for the instrument.
- **Auto sign out (*Administrator profile only*)**
  Use the toggle to enable/disable automatic sign out of a user when no activity is detected on the instrument for a selected period of time.

Maintenance & services

Select **Maintenance & Services** to set the following instrument parameters.

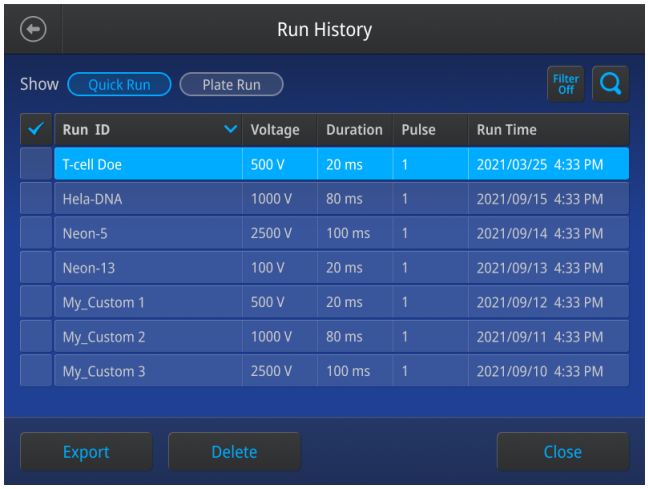


- (Administrator only) Select **Software Update** to update the System firmware. See “Upgrade the system firmware” on page 61 for instructions on updating the firmware.
- (Administrator only) Select **Self Verification Test** to conduct a check on the instrument hardware. The check includes testing the pumps, motors, and other components. See “Maintenance” on page 60 for instructions on conducting the self-verification test.
- (Administrator only) Select **Export Instrument Log** to export the instrument logs to a USB memory device. Insert the USB memory device into the USB port before using this feature.
- (Administrator only) Select **Restore factory settings** is used to reset the instrument to the original factory settings See “Restore factory settings (Administrator profile only)” on page 65.

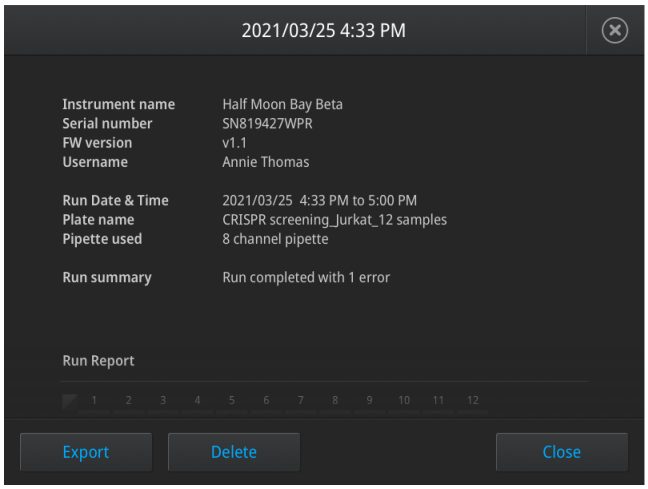
Run History

Select **Run History** to display the entire list of runs performed by the instrument.

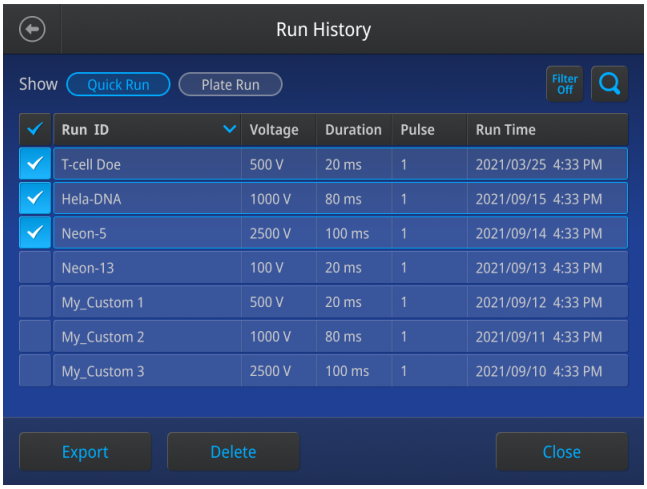
- Select a particular file to view the details of that run.



- Select **Export** to save the run details to a USB memory device, or **Delete** to delete the the run history file.



- Select **Manage** to perform the following functions:



- Delete a run report
- Export a run report

D

Specifications

Product specifications

Operating power:	100–240 VAC, Frequency 50/60 Hz, 270 VA
Output:	0.5-2.5 kV
Pulse width:	1-100 ms
Maximum duty cycle:	0.1
Charging time:	Maximum 6 seconds
Altitude:	Up to 2,000 meters
Operating temperature:	15℃ to 30℃
Maximum relative humidity:	Up to 80%
Degree of protection:	IPX0
Protective earthing:	Class I (earthed)
Installation category:	II
Instrument type:	Benchtop unit
Pulse generator dimensions:	9.5 inches (w) × 10.1 inches (l) × 7.6 inches (h)
Pulse generator weight:	10.1 pounds (4.6 kg)
Pulse generator built-in features:	Touch screen (800 × 600 pixels), digital display
Single-channel pipette station dimensions:	4.9 inches (w) × 6.6 inches (l) × 5 inches (h)
8-channel pipette station dimensions:	5.2 inches (w) × 8.9 inches (l) × 4.8 inches (h)

Networking requirements

Configure the system behind a firewall. If outbound traffic is limited, the following firewall exceptions are required to support system features:

Firewall lexception requirements			
URL	**Port**	**Purpose**	**Applies to…**
*.instrumentconnect.com	outbound 443	To support instrument management and identify	Thermo Fisher™ Connect Platform only
*.thermofisher.com	outbound 443	To support instrument management and identify	Thermo Fisher™ Connect Platform LAN connection
*.s3-us-east-1.amazonaws.com	outbound 443	To allow connection to the Thermo Fisher™ Connect Platform	Thermo Fisher™ Connect Platform only
*.iot-us-east-1.amazonaws.com	outbound 443	To allow connection to the Thermo Fisher™ Connect Platform	Thermo Fisher™ Connect Platform only
Allowed port requirements			
—	7443	To support instrument discovery Uses multicast address 224.0.0.251	LAN connection Direct connection
—	TCP 445 (SMB v3 or higher)	To support file sharing	LAN connection Direct connection

E Related products

Accessory products

Additional products

The following products are for use with the Neon™ NxT Electroporation System and are available separately.

For more information, go to **thermofisher.com** or contact Technical Support.

Catalog numbers that appear as links open the web pages for those products.

Product	Quantity	Catalog no.
Neon™ NxT Kit, 10 µL, with 1-Channel Tubes	1 kit (50 reactions)	N1025
	1 kit (192 reactions)	N1096
Neon™ NxT Kit, 100 µL, with 1-Channel Tubes	1 kit (50 reactions)	N10025
	1 kit (192 reactions)	N10096
Neon™ NxT 1-Channel Pipette	1 each	NEON1P
Neon™ NxT 1-Channel Pipette Station	1 each	NEON1PS
Neon™ NxT 1-Channel Tubes	1 pack of 96 tubes	NT96
	1 pack of 24 tubes	NT24
Neon™ NxT Kit, 10 µL, with 8-Channel Tubes	1 kit (192 reactions)	N1096-8
	1 kit (768 reactions)	N10384-8
Neon™ NxT Kit, 100 µL, with 8-Channel Tubes	1 kit (192 reactions)	N10096-8
	1 kit (768 reactions)	N100384-8
Neon™ NxT 8-Channel Pipette	1 each	NEON8P
Neon™ NxT 8-Channel Pipette Station	1 each	NEON8PS
Neon™ NxT 8-Channel Tubes	1 pack of 8 tubes	NT8
Neon™ NxT Electroporation System 8-Channel Upgrade Package	1 each	NEON8 NEON8U
Products for increasing scale of electroporation		
CTS™ Xenon™ Electroporation Instrument	1 instrument	A52727

(continued)

Product	Quantity	Catalog no.
CTS™ Xenon™ SingleShot Electroporation Chamber	6 chambers	A50305
CTS™ Xenon™ MultiShot Electroporation Cartridge	1 cartridge	A50306
CTS™ Xenon™ Electroporation Buffer	100 mL	A4997901 A4997902
CTS™ Xenon™ Genome Editing Buffer	100 mL	A4998001 A4998002
Products for cell processing and isolation		
CTS™ Rotea™ Counterflow Centrifugation System	1 system	A50760 A44769
CTS™ DynaMag™ Magnet	1 each	12102
CTS™ DynaCellect™ Magnetic Separation System	1 system	A55867
CTS™ Rotea™ Single-Use Kit	10 kits 5 kits	A49585 A49313
CTS™ Rotea™ Hi-Flow Single-Use Kit	10 kits 5 kits	A46575 A49239
CTS™ AIM-V™ Medium, without phenol red, without antibiotics	2 L	A4672701
Tube clamps for CTS™ Rotea™ Single-Use Kit	100 clamps	A49127
Sterilized sample ports for CTS™ Rotea™ Single-Use Kit	10 ports	A50111
Countess™ 3 FL Automated Cell Counter	1 instrument	AMQAX2000
TruBio™ Discovery Automation System Bundle, 50 L HyPerforma Rocker Bioreactor, load cells	1 bundle	TBD1-HPRK-LC
Herasafe™ 2030i Class 2 A2 Biological Safety Cabinets	1 unit	51032335 51032334
Heracell™ VIOS™ 160i CR Incubator	1 unit	51033770 51033775
Sorvall™ X4R Pro Centrifuge	1 unit	75009018
SL4 Plus Centrifuge	1 unit	75016086

(continued)

Product	Quantity	Catalog no.
Dynabeads™ Untouched™ Human T Cells Kit	1 kit	11344D
Dynabeads™ Human T-Activator CD3/CD28 for T cell expansion and actviation	2 mL 0.4 mL 5 × 2 mL	11131D 11161D 11132D
Dynabeads™ Mouse T-Activator CD3/CD28 for T cell expansion and actviation	0.4 mL 2 mL 5 × 2 mL	11452D 11456D 11453D
Dynabeads™ Untouched™ Human B Cells Kit	1 kit	11351D
Dynabeads™ Untouched™ Human CD4 T Cells Kit	1 kit	11346D 11352D
DynaMag™-2 Magnet	1 unit	12321D
DynaMag™-15 Magnet	1 unit	12301D
DynaMag™-5 Magnet	1 unit	12303D
DynaMag™-50 Magnet	1 unit	12302D
DynaMag™-96 Side Magnet	1 unit	12331D
Dynabeads™ Regulatory CD4+/CD25+ T Cell Kit	1 kit	11363D
Dynabeads™ Untouched™ Human NK Cells Kit	1 kit	11349D
Dynabeads™ CD34 Positive Isolation Kit	1 kit	11301D
Dynabeads™ CD4 Positive Isolation Kit	1 kit	11331D
Payloads for gene editing		
TrueCut™ HiFi Cas9 Protein (1 µg/µL)	10 µg 25 µg	A50574 A50575
TrueCut™ HiFi Cas9 Protein (5 µg/µL)	500 µg 100 µg	A50577 A50576
TrueCut™ Cas9 Protein v2	100 µg 25 µg 10 µg 500 µg	A36498 A36497 A36496 A36499

(continued)

Product	Quantity	Catalog no.
CTS™ TrueCut™ Cas9 Protein	2.5 mg 5.0 mg	A45220 A45221
TrueGuide™ sgRNA Positive Control, HPRT1 (human)	3 nmol	A35524
TrueGuide™ sgRNA Negative Control, non-targeting 1	3 nmol	A35526
TrueGuide™ sgRNA Positive Control, AAVS1 (human)	3 nmol	A35522
TrueTag™ Donor DNA Kit, GFP	1 kit	A42992
Precision gRNA Synthesis Kit	1 kit	A29377
GeneArt™ CRISPR Nuclease mRNA	15 µg	A29378
TrueTag™ Donor DNA Kit, RFP	1 kit	A42993
TrueTag™ Knockout Enrichment Donor DNA Kit	1 kit	A53815
FlexCut™ TALEN mRNA Pairs	custom	A4688
TrueTag™ Donor DNA Kit, HA	1 kit	A53809
TrueTag™ Donor DNA Kit, BFP stem	1 kit	A53814
TrueTag™ Donor DNA Kit, 6xHis	1 kit	A53811
TrueTag™ Donor DNA Kit, GFP stem	1 kit	A53812
TrueTag™ Donor DNA Kit, Myc	1 kit	A53810
TrueTag™ Donor DNA Kit, YFP	1 kit	A53807
TrueTag™ Donor DNA Kit, DDK	1 kit	A53808
GeneArt™ CRISPR Nuclease Vector with OFP Reporter Kit	1 kit	A21174 A21178
GeneArt™ CRISPR Nuclease Vector with CD4 Enrichment Kit	1 kit	A21175 A21177
TrueGuide™ Synthetic sgRNA	custom	**thermofisher.com/us/en/home/life-science/genome-editing/crispr-libraries/trueguide-grnas.html**

(continued)

Product	Quantity	Catalog no.
LentiArray™ Lentiviral sgRNA	custom	**thermofisher.com/us/en/home/life-science/genome-editing/geneart-crispr/crispr-libraries/lentiarray-crispr-libraries/lentiarray-grnas.html**
TrueGuide™ gRNA	custom	**thermofisher.com/trueguide**
TrueGuide™ sgRNA Positive Control, Rosa26 (mouse)	3 nmol	A35525
TrueGuide™ sgRNA Positive Control, CDK4 (human)	3 nmol	A35523
LentiArray™ Cas9 Lentivirus	100 µL 1 mL	A32064 A32069
Products for cell culture		
IMDM	500 mL 1 L	12440053 12440046
CTS™ NK-Xpander™ Medium	5 L	A5019002
CTS™ DPBS with calcium, magnesium , bag format	2 L	A4737901
CTS™ DPBS without calcium chloride, without magnesium chloride	2 L	A1285602
DMEM, high glucose, GlutaMAX™ Supplement, pyruvate	500 mL 5 L	10569010 10569069
DMEM, no glucose	500 mL	11966025
DMEM, high glucose	500 mL 5 L	11965092 11965167
RPMI 1640 Medium, no glucose	500 mL	11879020
RPMI 1640 Medium, GlutaMAX™ Supplement	500 mL	61870036
StemPro™-34 SFM (1X)	500 mL	10639011
CTS™ OpTmizer™ T-Cell Expansion SFM	1 L	A1048501
CTS™ OpTmizer™ T-Cell Expansion SFM, no phenol red	1 L	A3705001
CTS™ OpTmizer™ Pro Serum Free Medium	1 L	A4966101
CTS™ Immune Cell SR	50 mL	A2596101

(continued)

Product	Quantity	Catalog no.
StemPro™ hESC SFM	1 kit	A1000701
KnockOut™ Serum Replacement	500 mL	10828028
CTS™ GlutaMAX™-I Supplement	100 mL	A1286001
CTS™ KnockOut™ DMEM/F12	500 mL	A1370801
Fetal Bovine Serum, dialyzed, US origin	500 mL	26400044
Products for cell analysis		
Attune™ CytPix™ Flow Cytometer	1 unit	A51849
Attune™ NxT Flow Cytometer	1 unit	A29004
CytKick™ Autosampler	1 unit	A42901
CytKick™ Max Autosampler	1 unit	A51849
Luminex™ xMAP™ INTELLIFLEX System	1 system	APX2020
Luminex™ 200™ Instrument System	1 system	APX10031
Luminex™ FLEXMAP™ Instrument System	1 system	APX1342
Varioskan™ LUX Multimode Microplate Reader	1 instrument	VL0000D0 VLB000GD0
Multiskan™ SkyHigh Microplate Spectrophotometer	1 instrument	A51119500C A51119600C
Multiskan™ FC Microplate Photometer	1 instrument	1410101
CellInsight™ CX7 LZR Pro High Content Screening Platform	1 each	HCSDCX7LZRPRO
EVOS™ M7000 Imaging System	1 system	AMF7000
QuantStudio™ 6 Flex Real-Time PCR System	1 system	4485699 4485697 4485691
7500 Fast Real-Time PCR Instrument	1 instrument	4351106
QuantStudio™ 6 Pro Real-Time PCR System	1 system	A43167 A43160 A43161

(continued)

Product	Quantity	Catalog no.
QuantStudio™ 7 Pro Real-Time PCR System	1 system	A43163 A43170 A43183
QuantStudio™ Absolute Q™ Digital PCR System	1 system	A52627
BigDye™ Terminator v3.1 Cycle Sequencing Kit	1 kit	4337454 4337455 4337458
SeqStudio™ 8 Flex Genetic Analyzer	1 instrument	A53627
Genexus™ Integrated Sequencer	1 instrument	A45727
Ion Chef™ Instrument	1 instrument	4484177
HID Ion Chef™ Instrument	1 instrument	A30070
GeneChip™ Scanner 3000 7G System	1 instrument	00-0210
GeneTitan™ MC Instrument	1 instrument	00-0373E
Ion GeneStudio™ S5 System	1 instrument	A38194
3500xL Genetic Analyzer	1 instrument	4406016
ABI PRISM™ 3100 Genetic Analyzer	1 instrument	4359571
3730xL DNA Analyzer	1 instrument	A41046
SeqStudio™ Genetic Analyzer	1 instrument	A46367
iBlot™ 2 Gel Transfer Device	1 unit	IB21001
iBlot™ 2 Transfer Stacks, nitrocellulose, regular size	10 stacks	IB23001
iBright™ FL1500 Instrument	1 instrument	A44241 A44115
XCell™ SureLock™ Mini-Cell™	1 unit	EI0001
Mini Gel Tank	1 unit	A25977
Power Blotter XL System	1 unit	PB0013
Mini Blot Module	1 unit	B1000
GeneArt™ Genomic Cleavage Detection Kit	1 kit	A24372

(continued)

Product	Quantity	Catalog no.
ProcartaPlex™ Multiplex Immunoassay Kits	custom	**thermofisher.com/us/en/home/life-science/antibodies/immunoassays/procartaplex-assays-luminex/procartaplex-immunoassays.html**
ProQuantum™ High-sensitivity Immunoassay Kits	custom	**thermofisher.com/immunoassays/elisa/query/proquantum**
QuantiGene™ Plex Gene Expression Assays	custom	**thermofisher.com/us/en/home/life-science/gene-expression-analysis-genotyping/quantigene-rna-assays/quantigene-singleplex-assay/quantigene-singleplex-assay-ordering**
eBioscience™ Human Regulatory T Cell Staining Kit	1 kit	88-8999-40
eBioscience™ Antibodies	custom	**thermofisher.com/us/en/home/life-science/antibodies/ebioscience.html**

F

Safety

Safety information

Follow the instructions in this section to ensure safe operation of the Neon™ NxT device. The Neon™ NxT Electroporation System is designed to meet EN61010-1 Safety Standards. To ensure safe, reliable operation, always operate the Neon™ NxT Electroporation System according to the instructions in this manual. Failure to comply with the instructions in this manual may create a potential safety hazard, and will void the manufacturer's warranty and void the EN61010-1 safety standard certification. Life Technologies is not responsible for any injury or damage caused by use of this instrument when operated for purposes which it is not intended. All repairs and service should be performed by Life Technologies.

- Always ensure that the power supply input voltage matches the voltage available in your location.
- For operating environment, see "Environmental requirements" on page 97.
- This device is air-cooled so its surfaces become hot during operation. When installing the device, leave a space of more than 10 cm (4 inches) around it.
- Never insert metallic objects into the air vents of the device as this could result in electrical shock, personal injury and equipment damage.
- Always set the main switch on the power supply unit to OFF before connecting the power cord to the wall outlet.
- Always ensure that the grounding terminal of the device and that of the wall outlet are properly connected. Connect the power cord to a grounded, 3-conductor power outlet.
- 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 the unit.
- Be sure to set the main switch to OFF, unplug the power cord, and secure the pipette station before moving the device.

Informational symbols

Symbol and description
CAUTION! Risk of danger. Consult the manual for further safety information.
CAUTION! Risk of electrical shock.

(continued)

Symbol and description	
	WEEE (Waste Electrical and Electronic Equipment) symbol indicates that this product should not be disposed of in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of WEEE. This instrument meets European requirement WEEE Directive 2012/19/EU.
	**ON** (power)
	OFF (power)
	Protective earth (ground)
	The CE mark symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the Neon™ NxT Electroporation System is subject to the conditions described in this manual. The protection provided by the device may be impaired if the instrument is used in a manner not specified by the manufacturer.
	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.” Instruments bearing the TUV symbol are certified by TUV Product Services to be in conformance with the applicable safety standard for the US and Canada.
	The UKCA mark symbolizes that the product conforms to all applicable provisions in Great Briitain (England, Wales, and Scotland) for which this marking is required. Operation of the Neon™ NxT Electroporation System is subject to the conditions described in this manual. The protection provided by the device may be impaired if the instrument is used in a manner not specified by the manufacturer.
	China RoHS EFUP 25

Informations de sécurité

Suivez les instructions de cette section pour vous assurer d'utiliser l'appareil Neon™ NxT en toute sécurité. Le Neon™ NxT Electroporation System est conçu pour répondre aux normes de sécurité EN61010-1. Pour assurer un fonctionnement sûr et fiable, utilisez toujours le Neon™ NxT Electroporation System conformément aux instructions de ce manuel. Le non-respect des instructions contenues dans ce manuel pourrait engendrer un éventuel danger pour la sécurité et annulerait la garantie du fabricant ainsi que la certification à la norme de sécurité NF EN61010-1. Life Technologies ne peut être tenu responsable de toute blessure ou dommage provoqués par l'utilisation de cet instrument dans des buts autres que ceux prévus. Toutes les réparations et la maintenance doivent être effectuées par Life Technologies.

- Assurez-vous toujours que la tension d'entrée de l'alimentation corresponde à la tension disponible sur le lieu d'utilisation.
- Pour l'environnement d'exploitation, consultez la page "Environmental requirements" on page 97.
- Cet appareil étant aéroréfrigéré, ses surfaces chauffent lorsqu'il fonctionne. Lors de l'installation de l'appareil, laissez un espace supérieur à 10 cm (4 pouces) autour de celui-ci.
- N'introduisez jamais d'objets métalliques dans les orifices d'aération de l'appareil, car cela pourrait provoquer un choc électrique, des blessures corporelles ou endommager l'équipement.
- Mettez toujours le commutateur principal de l'alimentation sur OFF (ARRÊT) avant de brancher le cordon d'alimentation sur la prise murale.
- Vérifiez toujours que la borne de mise à la terre de l'appareil et celle de la prise murale sont correctement raccordées. Branchez le cordon d'alimentation sur une prise d'alimentation à 3 conducteurs et reliée à la terre.
- Pour éviter tout risque potentiel de choc électrique, vérifiez que le cordon d'alimentation est correctement relié à la terre.
- Veillez à placer l'instrument de manière à pouvoir le débrancher facilement.
- Veillez à mettre le commutateur principal sur OFF (ARRÊT), à débrancher le cordon d'alimentation et à immobiliser la station à pipettes avant de déplacer l'appareil.

Informational symbols

Symbol and description	
	**MISE EN GARDE !** Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité.
	**MISE EN GARDE !** Risque de choc électrique.
	Le symbole **DEEE** (Déchets d'équipements électriques et électroniques) indique que ce produit ne doit pas être mis au rebut avec des déchets ménagers non triés. Suivez la réglementation locale relative à l'élimination des déchets usuels pour réduire l'impact environnemental des DEEE. Rendez-vous sur **www.invitrogen.com/weee** pour prendre connaissance des options de collecte et de recyclage..

(continued)

Symbol and description	
	**ON (MARCHE)** (alimentation)
	OFF (ARRÊT) (alimentation)
	Protection par la mise à la terre (masse)
	La marque CE est un symbole indiquant que le produit est conforme à toutes les dispositions applicables de la Communauté européenne pour lesquelles ce marquage est obligatoire. L'utilisation du Neon™ NxT Electroporation System est soumise aux conditions décrites dans ce manuel. Si vous utilisez l'instrument d'une manière non spécifiée par le fabricant, la protection offerte par l'appareil pourrait s'en trouver détériorée.
	Ce produit est conforme à UL 61010-1, CAN/CSA C22.2 No.61010-1 «Exigences de sécurité pour l'équipement électrique pour la mesure, le contrôle et l'utilisation en laboratoire, Partie I : Généralité Les exigences.» Les instruments portant le symbole TUV sont certifiés par TUV Product Services conforme à la norme de sécurité applicable aux États-Unis et au Canada.
	La marque UKCA est un symbole indiquant que le produit est conforme à toutes les dispositions applicables en Grande-Bretagne (Angleterre, Pays de Galles et Écosse) pour lesquelles ce marquage est obligatoire. L'utilisation du Neon™ NxT Electroporation System est soumise aux conditions décrites dans ce manuel. Si vous utilisez l'instrument d'une manière non spécifiée par le fabricant, la protection offerte par l'appareil pourrait s'en trouver détériorée.
	La marque de conformité réglementaire indique qu'elle est conforme aux normes australiennes compatibilité électromagnétique
	Chine RoHS EFUP 25

Environmental requirements

Condition	Acceptable Range
Installation site	Indoor use only
Electromagnetic interference	Do not use this device in close proximity to sources of strong electromagnetic radiation (for example, unshielded intentional RF sources). Strong electromagnetic radiation may interfere with the proper operation of the device.
Altitude	Maximum of 2000 m (6500 ft.) above sea level
Operating conditions	Temperature: 15 to 30°C (59 to 86°F) **Note:** For optimal performance, avoid rapid or extreme fluctuations in room temperature.
Storage and transport conditions	• Humidity: 20–80% relative humidity (noncondensing) • Temperature: –30 to 60°C (–22 to 140°F)
Vibration	Ensure that the instrument is not adjacent to strong vibration sources, such as a centrifuge, pump, or compressor. Excessive vibration will affect instrument performance.
Pollution degree	The instrument has a Pollution Degree rating of II. The instrument may only be installed in an environment that has nonconductive pollutants such as dust particles or wood chips. Typical environments with a Pollution Degree II rating are laboratories and sales and commercial areas. The noise output of the instrument is ≤45 dB(A) when running.
Other conditions	Ensure the instrument is located away from any vents that could expel particulate material onto the instrument components. Avoid placing the instrument adjacent to heaters, cooling ducts, or in direct sunlight.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.

Safety

Reference	Description
EU Directive 2014/35/EU	European Union “Low Voltage Directive”
IEC 61010-1 EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1	*Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements*
IEC 61010-2-081 EN 61010-2-081 UL 61010-2-081 CAN/CSA C22.2 No. 61010-2-081	*Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes*

EMC

Reference	Description
Directive 2014/30/EU	European Union “EMC Directive”
IEC 61326-1 EN 61326-1	*Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements*
FCC Part 15 Subpart B (47 CFR)	U.S. Standard Radio Frequency Devices
ICES-003	*Information Technology Equipment (Including Digital Apparatus) – Limits and Methods of Measurement:*

Class A notice

This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications.

Environmental design standards

Reference	Description
EU Directive 2012/19/EU	European Union “WEEE Directive”—Waste electrical and electronic equipment
EU Directive 2011/65/EU Commission Delegated Directive (EU) 2015/863	European Union “RoHS Directive”—Restriction of hazardous substances in electrical and electronic equipment
Regulation EC 1907/2006	European Union “REACH Directive”—Registration, Evaluation, Authorisation and Restriction of Chemicals
SJ/T 11364-2014	“China RoHS” Standard — Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products

Electrical safety

The following information on electrical safety must be observed, failing to follow these instruction may result in electric shock, fire and/or serious personal injury or death.

Service operation requirements

In the event of an equipment malfunction, it is the responsibility of the customer to report the need for service to Thermo Fisher Scientific or to one of the authorized agents. For service information, contact Technical Support (“Customer and technical support” on page 103).

Servicing of this device is to be performed by trained service pe rsonnel only.

- Prior to switching on the product, ensure that the nominal voltage setting on the product matches the nominal voltage of the AC supply network.
- This product should be connected to the power mains using a 3-wire (two conductors and ground) power cable and plug. Use this power cable with a properly grounded electrical outlet to avoid electrical shock.
- If extension cords or connector strips are implemented, they must be checked on a regular basis to ensure that they are safe to use.
- The appliance coupler of the connecting cable is regarded as the disconnecting device. In such cases, always ensure that the power plug is easily reachable and accessible at all times (corresponding to the length of connecting cable, approx. 2 m).
- Never use the product if the power cable is damaged. Check the power cable on a regular basis to ensure that it is in proper operating condition. By taking appropriate safety measures and carefully laying the power cable, you can ensure that the cable will not be damaged and that no one can be hurt by, for example, tripping over the cable or suffering an electric shock.
- Do not insert the plug into sockets that are dusty or dirty. Insert the plug firmly and all the way into the socket. Otherwise, sparks that result in fire and/or injuries may occur.
- Do not overload any sockets, extension cords or connector strips; doing so can cause fire or electric shocks.

- Ensure that the connections with information technology equipment, e.g. PCs or other industrial computers, comply with the IEC60950-1/EN60950-1, IEC61010-1/EN 61010-1, or IEC 62368-1/EN 62368-1 standards that apply in each case.
- Unless expressly permitted, never remove the cover or any part of the housing while the product is in operation. Doing so will expose circuits and components and can lead to injuries, fire or damage to the product.
- Use suitable overvoltage protection to ensure that no overvoltage (such as that caused by a bolt of lightning) can reach the product. Otherwise, the person operating the product will be exposed to the danger of an electric shock.
- The overvoltage protection should limit the magnitude of the overvoltage surge to 1kV between the any of the power line and ground.
- Any object that is not designed to be placed in the openings of the housing must not be used for this purpose. Doing so can cause short circuits inside the product and/or electric shocks, fire or injuries.
- Prior to cleaning the product, disconnect it completely from the power supply. Use a soft, non-linting cloth to clean the product. Never use chemical cleaning agents such as alcohol, acetone or diluents for cellulose lacquers.

Chemical safety

WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.
- **IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

WARNING! HAZARDOUS WASTE (from instruments). Waste produced by the instrument is potentially hazardous. Follow the guidelines noted in the preceding General Chemical Handling warning.

Biological hazard safety

WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.

WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
  cdc.gov/labs/bmbl
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
  who.int/publications/i/item/9789240011311

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest service and support information.

- Worldwide contact telephone numbers
- Product support information
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have questions, contact Life Technologies at **www.thermofisher.com/support**.