USER GUIDE

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by life technologies™

Neon® Transfection System

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

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Contents

Product contents

Upon receiving the device

Examine the unit carefully for any damage incurred during transit. Any damage claims must be filed with the carrier. The warranty does not cover in-transit damage. To register the device, activate your warranty, and be notified of important updates, go to **www.lifetechnologies.com/neon**.

Neon® Transfection System

The contents of the Neon® Transfection Systems are listed below. The Neon® Transfection System is shipped at room temperature.

See page 3 for specifications and description of the Neon® Transfection System, and page 6 to set up the device.

Product	Quantity
Neon® Transfection Device	1
Specific Power Cord (for US/Canada/Taiwan/Japan, Europe, and UK)	4
Neon® Pipette	1
Neon® Pipette Station	1
Instruction Manual	1

Neon® Kits

The Neon® Kits are used with the Neon® Transfection Systems for efficient transfection of mammalian cells and are available separately from (page 40). The kits are available in two formats for electroporation of 10 µL and 100 µL samples.

The following components are included with the Neon® Kit. The Neon® Kits are shipped at room temperature.

Upon receipt, store the kit at room temperature. After use, store buffers at 4°C and all remaining kit components at room temperature.

Item	Neon® Kit, 10 µL		Neon® Kit, 100 µL	
	Cat. no. MPK1025 (50 reactions)	Cat. no. MPK1096 (192 reactions)	Cat. no. MPK10025 (50 reactions)	Cat. no. MPK10096 (192 reactions)
Neon® Tips	25 tips (10 µL)	96 tips (10 µL)	25 tips (100 µL)	96 tips (100 µL)
Neon® Tubes	5	20	5	20
Resuspension Buffer R (Proprietary)	1 mL	3 × 1 mL	10 mL	30 mL
Resuspension Buffer T (Proprietary)	1 mL	3 × 1 mL	10 mL	30 mL
Electrolytic Buffer E (Proprietary)	75 mL	2 × 150 mL	Not applicable	Not applicable
Electrolytic Buffer E2 (Proprietary)	Not applicable	Not applicable	75 mL	2 × 150 mL

Unpacking the Neon® Transfection System

Unpacking instructions

Follow the instructions below to unpack the Neon® Transfection System. The weight of the Neon® device is 13.2 pounds (6 kg).

1. Cut plastic tapes and remove the outer box. Save the outer box and other packaging material (in case you need to transport or ship the unit).
2. Remove the plastic bag from the top containing the manual, the Neon® Pipette box containing the pipette, and then remove the plastic bag containing the power cords from the box.
3. Remove the Neon® device and Neon® pipette station from the box and place on a flat, level surface.
4. Set up the Neon® Transfection System as described on page 6.

Neon® Transfection System

Front view

The front view of the Neon® device is shown below.

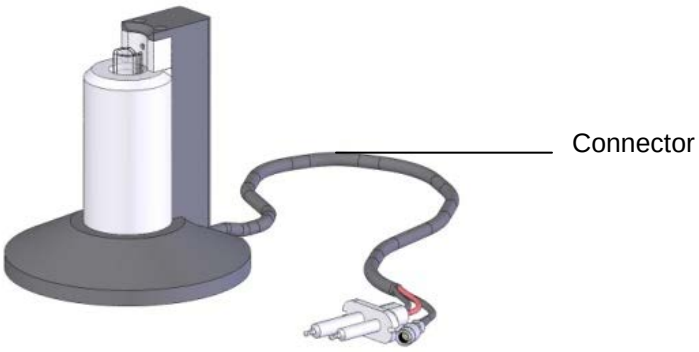
Rear view

The rear view showing various parts of the Neon® device are shown below.

The USB port (need to unscrew the panel to view the port) is used to connect a USB memory drive. The AC inlet is to connect to the power outlet on the wall, and high voltage and sensor port is to connect the high voltage and sensor connector of the Neon® Pipette Station to the unit.

Neon® Pipette Station

The Neon® Pipette Station is supplied with a high voltage and sensor connector which connects the pipette station to the Neon® device. The Neon® Pipette with a Neon® Tip and Neon® Tube is then used with the Neon® Pipette Station for electroporation of mammalian cells. The Neon® Pipette Station contains two electrodes.



User interface

The touchscreen user interface of the Neon® device consists of:

- The touchscreen buttons to operate the device
- The Digital Display that shows the protocol that is in use and various parameters of the protocol.

Introduction

About the product

Neon® Transfection System

The Neon® Transfection System is a novel, benchtop electroporation device that employs an electroporation technology by using the pipette tip as an electroporation chamber to efficiently transfect mammalian cells including primary and immortalized hematopoietic cells, stem cells, and primary cells.

The Neon® Transfection System efficiently delivers nucleic acids, proteins, and siRNA into all mammalian cell types including primary and stem cells with a high cell survival rate. The transfection is performed using as few as 1×10^4 or as many as 5×10^6 cells per reaction using a sample volume of 10 µL or 100 µL in a variety of cell culture formats (60 mm, 6-well, 48-well, and 24-well).

The Neon® Transfection System uses a single transfection kit (Neon® 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® Transfection System offers open and transparent protocols that are optimized for ease of use and simplicity. The Neon® device is preprogrammed with one 24-well optimization protocol to optimize conditions for your nucleic acid/siRNA and cell type, or you can program and store up to 50 cell-specific protocols in the Neon® device database. Optimized protocols for many commonly used cell types are also available on **www.lifetechnologies.com/neon** for your convenience to maximize transfection efficiencies for your cell types.

See page 3 for details on various parts of the system.

System components

The Neon® Transfection System consists of:

- Neon® Device

  The Neon® Device is a simple, user friendly benchtop electroporation device that employs the pipette tip as an electroporation chamber to efficiently transfect mammalian cells including primary and immortalized hematopoietic cells, stem cells, and primary cells. The device is preprogrammed with a 24-well optimization protocol and supports a database to store up to 50 user-specified protocols. See page 3 for details.

- Neon® Pipette Station

  The Neon® Pipette Station is a unique component of the system and holds the Neon® Pipette during electroporation and protects the user from any electrical shock exposures. The Neon® Tube which has an electrode near the bottom is inserted into the pipette station to transfer the electric field from the electrode inside the Neon® Tip. See page 3 for details.

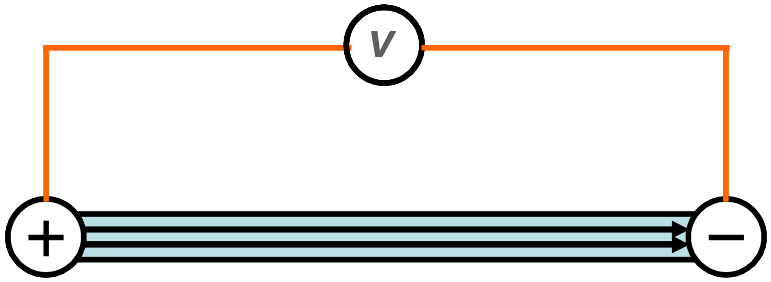
- Neon® Kits (not supplied with the device)

  The Neon® Kits contain the Neon® Tips, Neon® Tubes, and buffers for electroporation. The Neon® Kits are available in two formats for electroporation of 10 µL or 100 µL samples (page 40 for ordering information). See page 3 for details on Neon® Tips and Tubes.

System overview

Unlike standard cuvette based electroporation, the Neon® Transfection System uses a unique electroporation reaction chamber, the Neon® Tip that delivers a high electric field to the biological sample. The Neon® 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.

This next generation electroporation technology overcomes many of the limitations associated with standard cuvette based electroporation thereby increasing transfection efficiency and cell viability, and providing an ergonomic workflow.



The transfection occurs in the uniquely designed Neon® Tip using simple 3-step procedure.

1. Load a mixture of harvested cells and molecules to be delivered (e.g., DNA, RNA, siRNA) into the Neon® Tip.
2. Plug the Neon® Pipette with Neon® Tip into position in the Neon® Pipette Station with Neon® Tube; select your protocol on the device, and press **Start**.
3. Unplug the Neon® Pipette and transfer your transfected cells into a tissue culture vessel containing the appropriate medium.

Features

Important features of the Neon® Transfection System are listed below:

- User-friendly Neon® device benchtop design that easily fits in your tissue culture hood for easy, efficient transfection of a wide variety of mammalian cells including primary and stem cells
- Ability to transfect 1×10^4–5×10^6 cells per reaction in a sample volume of 10 µL or 100 µL in a variety of cell culture formats (60 mm, 6-well, 48-well, and 24-well)
- Utilizes a single buffer system for all cell types except primary suspension blood cells
- Simple touch screen interface for easy programming of electroporation parameters
- Available with one pre-programmed 24-well optimization protocol and the option to customize up to 50 cell specific protocols
- Built-in safety features in the device to enhance user safety

Description of parts

Neon® Device

The Neon® device is a simple, user friendly benchtop electroporation device. When used with a Neon® Pipette Station and Neon® Kits, the Neon® device efficiently transfects mammalian cells including primary and stem cells. The device is preprogrammed with a 24-well optimization protocol and supports a database to store up to 50 user-specified protocols.

See page vii for a front and rear view of the device.

Neon® Pipette

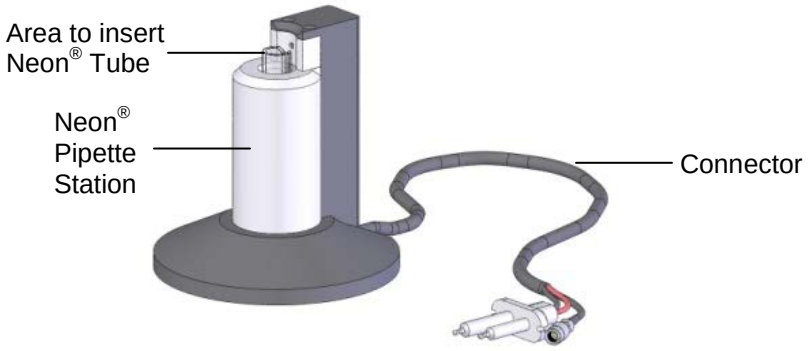
The Neon® Pipette utilizes a positive displacement pipette mechanism for pipetting mixtures containing cells and nucleic acid or siRNA. The Neon® Pipette is a fixed volume pipette and permanently calibrated at the manufacturing stage and does not require any further calibration.

The Neon® Pipette is designed for use with Neon® Tips only. Do not use any other tips with the Neon® Pipette.

Neon® Pipette Station

The Neon® Pipette Station is a unique component of the Neon® Transfection system. It holds a Neon® Pipette during electroporation procedures. The Neon® Pipette Station is equipped with many safety sensors and protection mechanisms that protect the user from any exposures to an electrical shock. The Neon® Pipette Station is connected to the Neon® device using the high voltage and sensor connector (see page 6 for details).

The Neon® Pipette Station also holds the Neon® Tube which has an electrode near the bottom that transfers the electric field from the electrode inside the Neon® Tip.



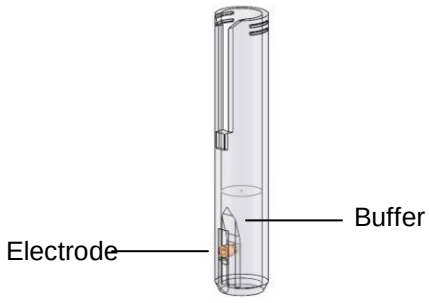
Neon® Tube

The Neon® Tube holds the Electrolytic Buffer during electroporation and is inserted into the Neon® Pipette Station. The Neon® Pipette with the Neon® Tip is then inserted into the Neon® Tube which has an electrode near the bottom that transfers the electric field from the electrode inside the Neon® Tip. The Neon® Tubes are supplied with Neon® Kits as well as available separately (page 40).

To avoid contamination, we strongly recommend using the tubes for a maximum of 10 times only. We recommend changing tube and buffer when switching to a different plasmid DNA/siRNA or cell type.

Tube Specifications:

Material:	Polystyrene
Capacity:	2.5–4 mL



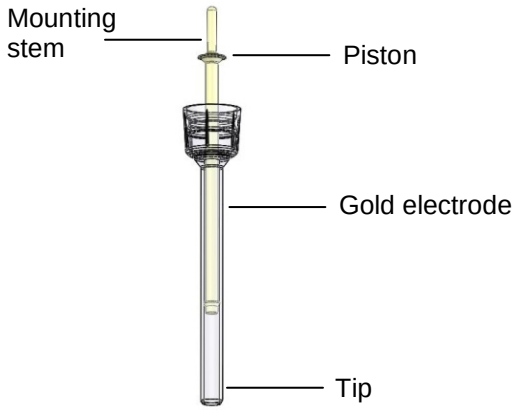
Neon® Tips

The Neon® Tips are disposable tips composed of a tip and piston used with the Neon® Pipette. The Neon® Tips contain a gold-plated electrode to create a disposable electric chamber for the delivery of a high electric field to biological samples. The Neon® Tips are supplied with Neon® Kits in two formats to support operating volumes of 10 µL and 100 µL, respectively (page 40 for ordering information).

To ensure repeatability and eliminate variation of the transfection conditions within or between experiments, we recommend that you do not use the Neon® Tip for 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.

Tip specifications:

Material:	Polypropylene
Capacity:	10 µL or 100 µL



Methods

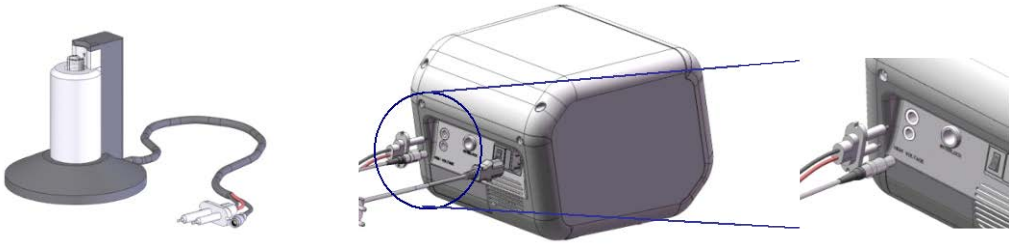
Getting started

Install the Neon® Device with Pipette Station

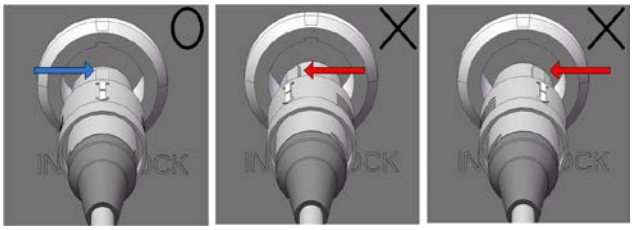
1. Unpack the Neon® device as instructed on page vi.
2. **Four power cords are shipped with the device to ensure that the cord you use is compatible with your local socket format.**
3. Place the Neon® device on a level laboratory bench. Keep the area around the unit clear to ensure proper ventilation of the unit.

   Note: The Neon® device has a small footprint and can be easily set up in the tissue culture hood for convenience.
4. **For your safety:** Position the device properly such that the **power** switch and AC inlet located on the rear of the unit (page vii) are easily accessible. Be sure to position the device such that it is easy to disconnect the unit.

   Note: Since Neon® 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.
5. Place the Neon® Pipette Station near the Neon® device.
6. Connect the high voltage and sensor connector on the Neon® Pipette Station to high voltage port and sensor port on the rear side of Neon® device, respectively.

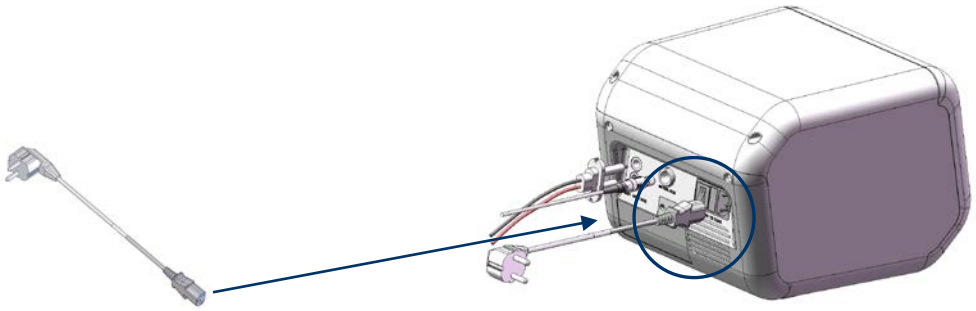


   Be sure to align the ridge indicated by a white arrow on the sensor connector on the Neon® Pipette Station with a groove indicated by a white dot on the sensor port of the Neon® device (see figure below for details).



   IMPORTANT! To connect or disconnect the sensor connector to the Neon® device, always handle the sensor connector using the cord plug and not the cord cable.
7. Ensure the AC power switch is in the **Off** position (page vii).

8. Attach the power cord to the AC inlet on the rear of the Neon® device and then to the electrical outlet. Use only properly grounded AC outlets and power cords.



9. To turn on the power, press the main power switch on the rear of the unit to **ON** position. The digital display shows start up screen (next page).
10. The Neon® device is operated by the touch screen on the front of the device. You can easily input electroporation parameters by lightly touching the touch screen with a finger tip or a touch screen pen. See next page for details.

You are ready to use the Neon® Transfection System. See page 14 for details.

Register the device

Visit **www.lifetechnologies.com/neon** to register the device and activate your warranty or extended warranty, and ensure that you receive product updates, special offers, and faster service.

Electroporation protocol options

There are three options to select an electroporation protocol for your cell type:

- If you already have the electroporation parameters for your cell type, input the parameters in the **Input Window** (see below).
- If you wish to add cell-specific electroporation parameters to the database on the device for future use, input the parameters in the **Database Window** (page 9). You can also view our library of protocols for commonly used cell types from **www.lifetechnologies.com/neon** and in put the parameters in the Database Window (see below) for various cell types.
- If you do not have any specific electroporation parameters for your cell type and wish to perform optimization, use the **Optimization Window** (page 11).

Input values limit

The Neon® device is designed to only input certain values and limits for each value are listed below. If your input value exceeds the maximum value, an error is displayed.

Input Voltage range: 500–2,500 V

Input Pulse Width range: 1–100 ms

Input Pulse Number range: 1–10

Input window

To create a cell specific protocol, if you already have the electroporation parameters for your cell type:

1. Press the power switch (located on the rear side of the unit, page vii) to turn **ON** the Neon® device. The unit checks to ensure that the Neon® Pipette Station is connected to the device and then the start up screen is displayed.

2. Press **Voltage** to activate the number key pad to input voltage value. Press the desired voltage value and press **Done** to save the value.

   Note: If any input value is out of the limit, an error message is displayed and the lowest value of limit is automatically set.

3. Press **Width** to activate the number key pad to input width value. Press the desired width value and press **Done** to save the value.
4. Press **Pulses** to activate the number key pad to input pulse value. Press the desired pulse value and press **Done** to save the value.
5. If you wish to save these electroporation parameters, press **Save** on the main screen to save the protocol in the database.
6. Press the desired protocol number button to edit the protocol. The selected protocol is highlighted.
7. Once the Edit screen is displayed, enter the **User** name by pressing the key pad buttons. The cursor automatically moves to the next field **Protocol** and is highlighted red.

   Continue to enter the information for **Voltage**, **Width**, and **Pulse.**
8. Press **Enter** to save the information in the database.
9. Proceed to preparing cells (pages 16–17) and DNA, and setting up the Neon® Pipette Station for electroporation (page 14).

Database window

Enter cell-specific protocols into the database. The database can store up to 50 cell-specific protocols.

1. Press the power switch (located on the rear side of the unit, page vii) to turn **ON** the Neon® device. The unit checks to ensure that the Neon® Pipette Station is connected to the device and then the start up screen is displayed.

2. Press **Database** button to start the database window. To scroll through the protocols in the database, use the right/left scroll buttons near the Database button.

The Database window shows:

- **Number** button: Indicates protocol number
- **User and Protocol**: Displays the user and protocol name
- Parameters (**Voltage, Width, Pulse**): Displays the electroporation parameter for each protocol
- Function buttons (**Load, Edit, and Delete**): Used to load, edit, or delete a protocol. The function buttons are activated only after a protocol is selected.
- **Page** scroll: To scroll to next or previous page

3. Press the desired protocol number button to edit the protocol. The selected protocol is highlighted.

Database window, continued

4. Once the Edit screen is displayed, enter the **User** name by pressing the key pad buttons. The cursor automatically moves to the next field **Protocol** and is highlighted red.

   Continue to enter the information for **Voltage**, **Width**, and **Pulse.**

   If you wish to password protect the protocol, enter the **Password** (up to 7 characters) and **Repeat Password** information using the key pad.

5. Press **Enter** to save the information in the database. To exit the edit screen without saving the parameters, press **X**.
6. The database window is displayed. Press the desired protocol and then press **Load** to load electroporation parameters from the database.
7. Proceed to preparing cells (pages 16–17) and DNA, and setting up the Neon® Pipette Station for electroporation (page 14).
8. To delete a protocol from the database, select the protocol by pressing the protocol number button. Press **Delete**. If the protocol in the database was password protected, a password screen is displayed. Enter the password and press **Enter** to delete the protocol.

Optimization window

Perform optimization of electroporation parameters using the preprogrammed 24-well optimization protocol. **These protocols are locked and cannot be edited.**

1. Press the power switch (located on the rear side of the unit, page vii) to turn **ON** the Neon® device. The unit checks to ensure that the Neon® Pipette Station is connected to the device and then the start up screen is displayed.

2. Press **Optimization** button to start the optimization window. To scroll through the protocols, use the right/left scroll buttons near the Optimization button.

   The Optimization window shows:

   - **Number** button: Indicates protocol number
   - **User and Protocol**: Displays the optimization and well number
   - Parameters (**Voltage, Width, Pulse**): Displays the electroporation parameter for each protocol
   - **Load** Function buttons: Used to load a protocol. The Load button is activated only after a protocol is selected.
   - **Page** scroll: To scroll to next or previous page

3. Press the desired protocol number button. The selected protocol is highlighted. Press **Load** to load the protocol. To exit the screen without loading the protocol, press **X**.

4. The electroporation parameters are displayed on the start up screen.
5. Proceed to preparing cells (pages 16–17) and DNA, and setting up the Neon® Pipette Station for electroporation (page 14).

Upgrade the firmware

Upgrades for the Neon® device firmware are available. To download Neon® device firmware upgrades, go to **www.lifetechnologies.com/neon**. Follow instructions on the page to download the upgrades.

General guidelines

Recommended kits

To use the Neon® device for electroporation of mammalian cells, you need to purchase the Neon® Kits. Ordering information is on page 40. **Do not** use any other kits with the unit.

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® Pipette and Tip correctly into the Neon® Pipette Station (see page 14 for details).
- Wear gloves, laboratory coat, and safety glasses during electroporation.
- Always use the Neon® device with Neon® Kits for electroporation of mammalian cells.
- The Neon® Transfection System is compatible for use with most mammalian cells including primary and stem cells.
- **Use high quality DNA and siRNA to obtain good transfection efficiency.**
- Follow the guidelines on pages 16–17 for cell preparation.
- Use an appropriate GFP (green fluorescent protein) construct or siRNA control (see next page for details) to determine transfection efficiency.
- Discard the Neon® Tips after 2 usages and Neon® Tubes after 10 usages as a biological hazard. We strongly recommend changing tube and buffer when switching to a different plasmid DNA/siRNA or cell type.
- Visit **www.lifetechnologies.com/neon** for a library of electroporation protocols for commonly used cell types.

Recommended buffers

The Neon® Kits contain two Resuspension Buffers. Use the appropriate Resuspension Buffer based on the cell type as below. The cell-specific Neon® transfection protocols available on **www.lifetechnologies.com/neon** indicate the type of Resuspension buffer for use with each cell type.

Resuspension Buffer R:

Use Resuspension Buffer R with **established adherent and suspension cell lines** such as 3T3-L1, HEK293, Cos7, C2C12, HeLa, HCT-15, PC-12, MDCK, Raw264.7, U-2OS, CEM, HL-60, K-562, Jurkat, LCL, Ramos, U-937, as well as **primary adherent cells** such as neuronal cells, stem cells, hepatocytes, HUVEC, macrophage cells, dendritic cells.

Resuspension Buffer T:

Use Resuspension Buffer T with **primary blood-derived suspension cells** such as T-cells, B-cells, NK cells, PBMC, monocytes.

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 (page 40) 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 initially assess transfection efficiency for your cell type using fluorescent microscopy, we recommend using a plasmid encoding GFP (green fluorescent protein) or any colored variant of GFP (Clontech or equivalent). For best results, the vector encoding the GFP should have the following features:

- Strong promoter active in a variety of mammalian cells such as the immediate early CMV (cytomegalovirus) promoter
- SV40 polyadenylation signals downstream of the GFP gene for proper processing of the 3' end of the GFP mRNA.
- Antibiotic selection marker
- pUC origin of replication for propagation in *E. coli*

siRNA control

For siRNA experiments, use BLOCK-iT™ Fluorescent Oligo for electroporation or *Silencer*® Select GAPDH Positive Control siRNA (page 40) to assess transfection efficiency.

Using the Neon® Transfection System

Introduction

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

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

See page 22 if you wish to use the preprogrammed optimization protocol.

Materials needed

Ordering information is on page 40.

- Cells
- Neon® Kits
- High quality DNA at a concentration of 1–5 µg/µL in deionized water or TE buffer, or high quality RNAi duplex at a concentration of 100–250 µM in nuclease-free water (page 13)
- Cell culture plates containing the appropriate medium
- D-PBS or Phosphate buffered saline (PBS) without Ca^{2+} and Mg^{2+} (page 40)
- Trypsin/EDTA or TrypLE™ Express (Cat. no. 12563) for adherent cells
- Countess® Automated Cell Counter (page 40) or equivalent

If you are a first time user of the Neon® Transfection System, we recommend that you review the protocol below and ensure that you are able to insert and use the Neon® Pipette and Tip correctly into the Neon® Pipette Station (see below for details) before you start using the system with your samples.

IMPORTANT!

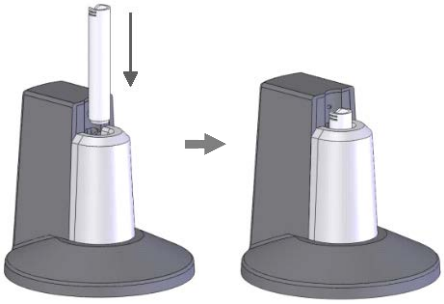
- To obtain the highest transfection efficiency and low non-specific effects, optimize transfection conditions by varying electrical parameters as described on page 22 using the pre-programmed optimization protocol in a 24-well format.
- Since the cell culture conditions vary from user to user, be sure to use low passage number, actively dividing cells (for dividing cells)
- For siRNA transfection, the concentration of RNAi duplex required will vary depending on the efficacy of the duplex. After the initial results, vary the siRNA final concentration from 10–200 nM.

  Note: The siRNA concentration in the Neon® transfection protocol refers to the siRNA concentration in the culture medium and not to the siRNA concentration in the electroporation mix in the Neon® Tip.

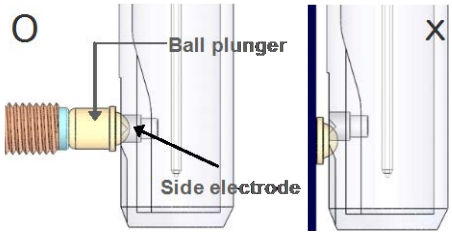
Set up the Neon® Pipette Station

1. Ensure the Neon® Pipette Station is connected to the Neon® device (page 6).
2. Fill the Neon® Tube with 3 mL of Electrolytic Buffer **(use Buffer E for 10 µL Neon® Tip and Buffer E2 for 100 µL Neon® Tip)**.

   Note: Make sure that the electrode on the side of the tube is completely immersed in buffer.
3. Insert the Neon® Tube into the Neon® Pipette Station until you hear a click sound.



Note: Make sure that the side electrode of the Neon® tube is well connected to the side ball plunger of the Neon® Pipette Station (see figure on the left below for correct position).



4. The station is ready for use. Proceed to preparing cells, next page.

Prepare adherent cells

1. Cultivate the required number of cells (see below).
2. One–two days prior to electroporation, transfer the cells into flask with fresh growth medium such that the cells are 70–90% confluent on the day of the experiment.

   5×10^4–2×10^5 cells per each 10 µL Neon® Tip for most optimized protocols.

   5×10^5–2×10^6 cells per each 100 µL Neon® Tip for most optimized protocols.
3. Pre-warm an aliquot of culture medium containing serum, PBS (without Ca^{2+} and Mg^{2+}), and Trypsin/EDTA solution to 37ºC.
4. Aspirate the media from cells and rinse the cells using PBS (without Ca^{2+} and Mg^{2+}).
5. Trypsinize the cells using Trypsin/EDTA or TrypLE Express (Cat. no. 12563).
6. After neutralization, harvest the cells in growth medium with serum (~0.75 mL for 10 µL Neon® Tip or 7.5 mL for 100 µL Neon® Tip).
7. Take an aliquot of trypsinized cell suspension and count cells to determine the cell density.
8. 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 minutes at room temperature.
9. Wash cells with PBS (without Ca^{2+} and Mg^{2+}) by centrifugation at 100–400 × g for 5 minutes at room temperature.
10. Aspirate the PBS and resuspend the cell pellet in Resuspension Buffer R at a final density of 1.0×10^7 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 (page 18) or optimization protocols (pages 24–29).
11. Prepare 24-well plates by filling the wells with 0.5 mL of culture medium containing serum and supplements **without antibiotics** and pre-incubate plates in a humidified 37°C/5% CO_2 incubator. If you are using other plate format, see page 18 for plating medium volume recommendations.

Prepare suspension cells

1. Cultivate the required number of cells (see below).
2. One to two days prior to electroporation, transfer the cells into flask with fresh growth medium such that the cells are 70–90% confluent on the day of the experiment. For most cell lines, the cell density is ~1–3× 10^6 cells/T-25 flask.

   1–5 × 10^5 cells per each 10 µL Neon® Tip for most optimized protocols.

   1–5 × 10^6 cells per each 100 µL Neon® Tip for most optimized protocols.
3. Pre-warm an aliquot (500 µL per sample for 10 µL Neon® Tips or 5 mL for 100 µL Neon® Tips) of culture medium containing serum. Also prepare an appropriate volume of PBS (without Ca^{2+} and Mg^{2+}).
4. Take an aliquot of cell culture and count the cells to determine the cell density.
5. Transfer the cells to a microcentrifuge tube or 15 mL conical tube and pellet the cells by centrifugation at 100–400 × g for 5 minutes at room temperature.
6. 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.
7. Aspirate the PBS and resuspend the cell pellet in Resuspension Buffer R or Resuspension Buffer T at a final density of 2.0 × 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 (page 18) or optimization protocols (pages 24–29).
8. Prepare 24-well plates by filling the wells with 0.5 mL of culture medium containing serum and supplements **without antibiotics** and pre-incubate plates in a humidified 37°C/5% CO_2 incubator. If you are using other plate format, see page 18 for plating medium volume recommendations.

Electroporation protocol

1. Make sure you have appropriate number of cells prepared as described on pages 16–17, have the plasmid DNA or siRNA at the suggested concentrations (page 13), and prepare a plate containing culture medium **without antibiotics** to transfer the electroporated cells.

   For details on optimizing the transfection efficiency of your cells, see page 22.

2. For each electroporation sample, the recommended amount of plasmid DNA or siRNA, cell number, and volume of plating medium **per well** are listed below. **Use Resuspension Buffer T for primary suspension blood cells.**

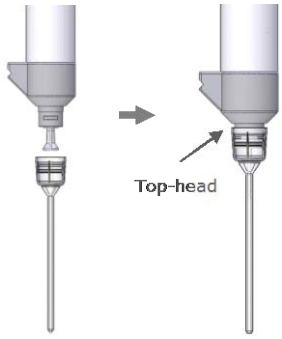
Format	Cell Type	DNA (µg)	siRNA (nM)	Neon® Tip	Vol. plating medium	Cell no.	Buffer R or Buffer T*
96-well	Adherent	0.25–0.5	10–200	10 µL	100 µL	$1–2 \times 10^4$	10 µL/well
	Suspension	0.5–1		10 µL		$2–5 \times 10^4$	10 µL/well
48-well	Adherent	0.25–1	10-200	10 µL	250 µL	$2.5–5 \times 10^4$	10 µL/well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

***Use Resuspension Buffer T for primary suspension blood cells.**

3. Set up a Neon® Tube with 3 mL Electrolytic Buffer (use Buffer E for 10 µL Neon® Tip and Buffer E2 for 100 µL Neon® Tip) into the Neon® Pipette Station (page 15).
4. Set the desired pulse conditions on the device based on your cell type (page 7).
5. Transfer the appropriate amount of plasmid DNA/siRNA into a sterile, 1.5 mL microcentrifuge tube.
6. Add cells to the tube containing plasmid DNA/siRNA and gently mix. See the above table for cell concentration, DNA, and plating volumes to use.
7. To insert a Neon® Tip into the Neon® Pipette, press the push-button on the pipette to the second stop to open the clamp.

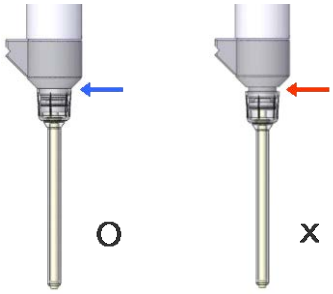
Electroporation protocol, continued

8. Insert the top-head of the Neon® Pipette into the Neon® Tip until the clamp fully picks up the mounting stem of the piston (see below)

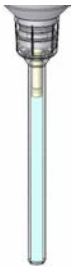


9. Gently release the push-button, continuing to apply a downward pressure on the pipette, ensuring that the tip is sealed onto the pipette without any gaps.

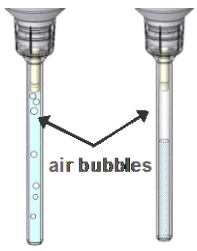
   Note: Ensure that the Neon® Pipette and Tip are tightly connected without a gap (see figure on the left) for trouble-free pipetting and proper electrical connection.



10. Press the push-button on the Neon® Pipette to the first stop and immerse the Neon® Tip into the cell-DNA/siRNA mixture. Slowly release the push-button on the pipette to aspirate the cell-DNA/siRNA mixture into the Neon® Tip.

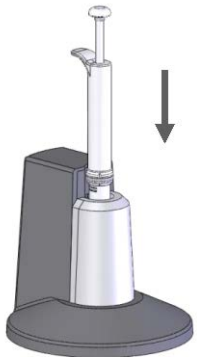


Note: Avoid air bubbles during pipetting as air bubbles cause arcing during electroporation leading to lowered or failed transfection. If you notice air bubbles in the tip, discard the sample and carefully aspirate the fresh sample into the tip again without any air bubbles.

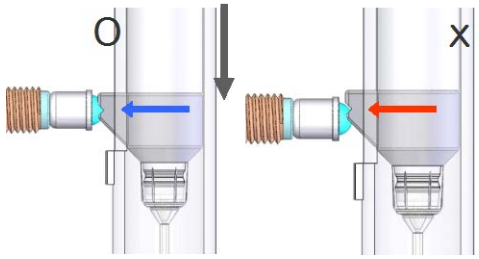


Electroporation protocol, continued

11. Insert the Neon® Pipette with the sample vertically into the Neon® Tube placed in the Neon® Pipette Station until you hear a click sound. Ensure that the pipette projection is inserted into the groove of the pipette station.



Note: Ensure the metal head of the Neon® Pipette is tightly connected to the ball plunger inside of the Neon® Pipette Station and to the Neon® Tube (see figure on the left for the correct position).



12. Ensure that you have selected the appropriate electroporation protocol and press **Start** on the touchscreen.
13. The Neon® device automatically checks for the proper insertion of the Neon® Tube and Neon® Pipette before delivering the electric pulse.

    Note: Monitor the Neon® Tip during electroporation to see if there is any arcing (sparks) that is caused by the presence of bubbles in the tip. Arcing results in low transfection efficiency and cell viability.
14. After delivering the electric pulse, **Complete** is displayed on the touchscreen to indicate that electroporation is complete.
15. Slowly remove the Neon® Pipette from the Neon® Pipette Station and immediately transfer the samples from the Neon® Tip by pressing the push-button on the pipette to the first stop into the prepared culture plate containing prewarmed medium.

    Note: We strongly recommend loading electroporated cells into growth medium **without antibiotics** that can greatly reduce the viability of your cells after transfection.
16. To discard the Neon® Tip, press push-button to the second stop into an appropriate biological hazardous waste container.

Electroporation protocol, continued

17. Repeat Steps 7–16 for the remaining samples.

    Be sure to change the Neon® Tips after using it twice and Neon® Tubes after 10 usages. Use a new Neon® Tip and Neon® Tube for each new plasmid DNA sample.

18. Gently rock the plate to assure even distribution of the cells. Incubate the plate at 37°C in a humidified CO_2 incubator.
19. If you are not using the Neon® device, turn the power switch on the rear to **OFF**.
20. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA).

Optimization

Based on your initial results, you may need to optimize the electroporation parameters for your cell type. See page 22 for using the 18-well or preprogrammed 24-well optimization protocol on the Neon® device.

Cleaning and maintenance

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

Important! Avoid spilling any liquid inside of the Neon® 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® 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® Pipette Station.** If the station does not work after drying, contact Technical Support (page 41).

For any other repairs and service, contact Technical Support (page 41). **Do not** perform any repairs or service on the Neon® device yourself as it is a high voltage hazard and to avoid any damage to the unit or voiding your warranty.

Optimization protocol for DNA and siRNA

Introduction

Electroporation is mainly dependent on the combination of three electric parameters such as the electric field, pulse width, and pulse number. Based on your initial results, you may need to optimize the electroporation parameters for your cell type especially the hard-to-transfect cells.

The Neon® device is preprogrammed with a 24-well optimization protocol using the 10 µL or 100 µL Neon® Tip that allows you to quickly optimize electric parameters for many adherent and suspension cell lines within days.

For primary blood suspension cells, use the 18-well optimization protocol with Resuspension Buffer T as described on page 26.

Materials needed

Ordering information is on page 40.

- Neon® 10 µL or 100 µL Kit
- Cells in Resuspension Buffer (prepared as described in pages 16–17)
- High quality DNA at a concentration of 1–5 µg/µL in deionized water or TE buffer or high quality RNAi duplex at a concentration of 100–250 µM in nuclease-free water (page 13)
- Cell culture plates containing the appropriate medium

Workflow

General workflow for optimization is described below. For detailed protocols, see the next page.

Optimization for plasmid

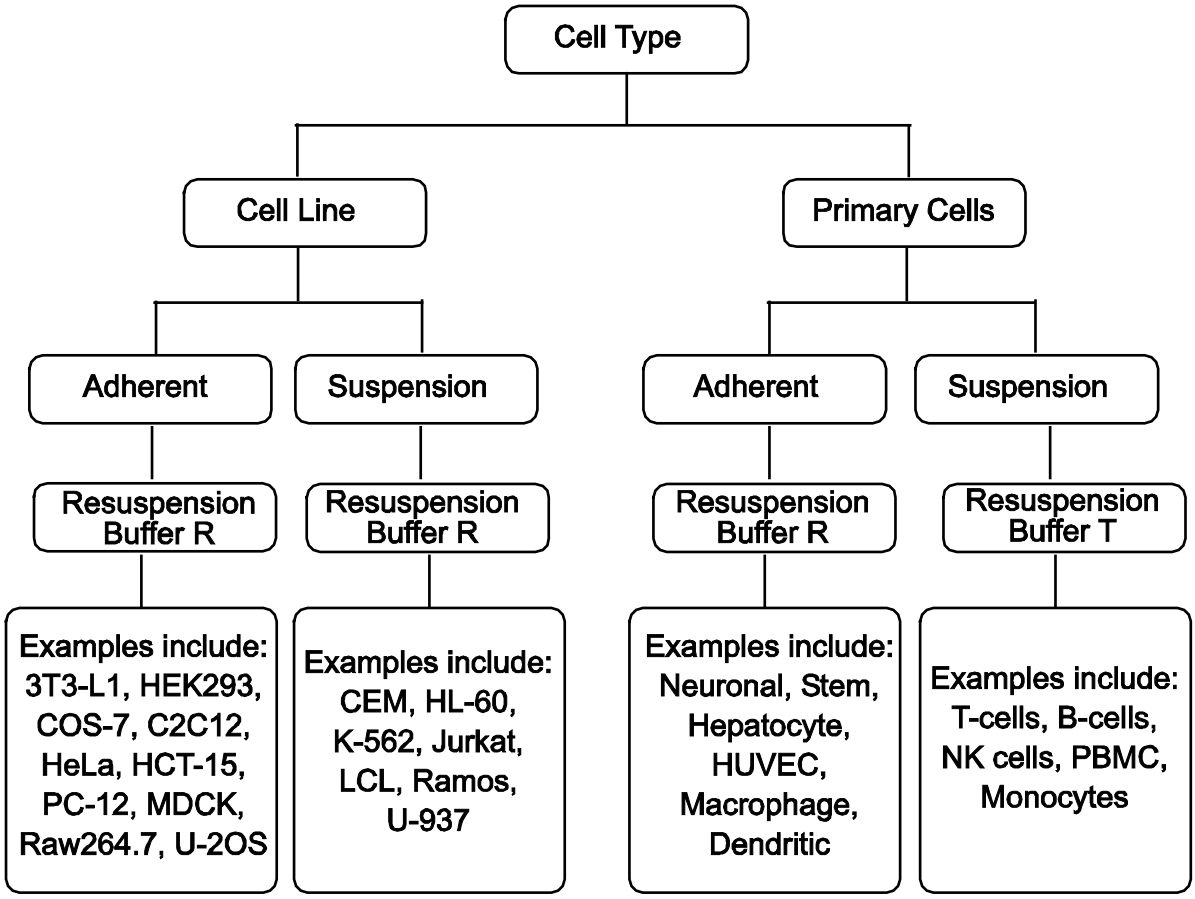
1. Perform 24-well optimization using the preprogrammed parameters.
2. Based on results from Step 1, perform optimization using narrower (bracket) parameters.
3. Based on results from Step 2, further refine the parameters to obtain optimal conditions (this is optional step).

Optimization for siRNA

1. Perform 24-well optimization using the preprogrammed parameters.
2. Based on results from Step 1, perform optimization using narrower (bracket) parameters.
3. Based on results from Step 2, perform optimization by varying siRNA final concentrations to 10 nM, 30 nM, 100 nM, and 200 nM.

Choose the appropriate optimization protocol

Based on your cell type, choose the appropriate optimization protocol as shown below. Optimizations are generally required for cell types which are not in the Neon® database but may also be needed for cell types that exist in the Neon® database as cell culture conditions may vary between laboratories.



24-well optimization protocol for adherent and suspension cell lines—Day One

1. Make sure you have cells prepared as described on pages 16–17, have the DNA or siRNA, and prepare a 24-well plate containing 0.5 mL culture medium with serum and **without antibiotics** to transfer the electroporated cells. Prepare enough cells and plasmid DNA/siRNA for at least 30 transfections.
2. For each electroporation sample using the **10 µL Neon® Tip** in **24-well** format, see table below. For using the 100 µL Neon® Tip in **24-well** format, adjust the amounts listed in the table below appropriately by 10-fold.

Cell type	Cell no.	DNA	siRNA	Resuspension Buffer R
Adherent	1×10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	10 µL/well 285 µL/plate
Suspension	2×10^5/well	1 µg DNA/well 30 µg/plate	100 pmol in 10 µL tip 200 nM per well	10 µL/well 270 µL/plate

3. Set up a Neon® Tube with 3 mL Electrolytic Buffer (use Buffer E for 10 µL Neon® Tip and Buffer E2 for 100 µL Neon® Tip) into the Neon® Pipette Station containing the cell-DNA/siRNA mixture as described on page 15.
4. Press **Optimization** and load the optimization protocols to begin electroporation using the parameters listed below.

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

24-well optimization protocol for adherent and suspension cell lines—Day One, continued

5. After electroporation, immediately remove the Neon® Pipette and transfer samples from the 10 µL Neon® Tip into prewarmed 0.5 mL culture medium.

   For 100 µL Neon® Tip, dilute samples 10-fold in 900 µL medium and transfer 100 µL of the sample to 0.4 mL prewarmed culture medium.
6. Repeat Steps 3–5 for the remaining samples.
7. Gently rock the plate to assure even distribution of the cells. Incubate the plate at 37°C in a humidified CO_2 incubator.
8. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA). Select the best conditions and proceed to the next day's experiment, page 27.

18-well optimization protocol for primary suspension blood cells—Day One

1. Make sure you have cells prepared as described on pages 16–17, have the DNA or siRNA, and prepare 18-wells of a 24-well plate containing 0.5 mL culture medium with serum and **without antibiotics** to transfer the electroporated cells. Prepare enough cells and plasmid DNA or siRNA for at least 20 transfections.
2. For each electroporation sample using the **10 µL Neon® Tip** in **18-wells of a 24-well plate**, see table below.

Cell type	Cell no.	DNA	siRNA	Resuspension Buffer T
Primary blood suspension cells	2×10^5/well	1 µg DNA/well 20 µg/plate	100 pmol in 10 µL tip 200 nM per well	10 µL/well 180 µL/plate

3. Set up a Neon® Tube with 3 mL Electrolytic Buffer E into the Neon® Pipette Station and Neon® Tip containing the cell-DNA/siRNA mixture.
4. Input the electroporation parameters in the Input window and perform electroporation using the parameters listed below.

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

5. After electroporation, immediately remove the Neon® Pipette and transfer samples from the 10 µL Neon® Tip into prewarmed 0.5 mL culture medium.
6. Repeat Steps 3–5 for the remaining samples.
7. Gently rock the plate to assure even distribution of the cells. Incubate the plate at 37°C in a humidified CO_2 incubator.
8. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA). Select the best conditions and proceed to the next day's experiment, next page.

Optimization protocol—Day Two

Select the best transfection conditions obtained from the previous experiment and fine-tune the optimization by narrowing the **Pulse Voltage**.

For example, if you obtained optimal conditions between 1,500 V, 20 ms and 1,400 V, 30 ms, (underlined in the table on the next page) perform optimization using these narrower parameters as below.

1. Make sure you have cells prepared as described on pages 16–17, have the DNA or siRNA, and prepare 18- or 24-wells of a 24-wells plate with 0.5 mL culture medium with serum and **without antibiotics** to transfer the electroporated cells.
2. For each electroporation sample using the **10 µL Neon® Tip**, see table below.

   For using the 100 µL Neon® Tip in **24-well** format, adjust the amounts listed in the table below appropriately by 10-fold.

Cell type	Format	Cell no.	DNA	siRNA	Resuspension Buffer
Adherent	24-well	1×10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	**Buffer R** 10 µL/well 285 µL/plate
Suspension	24-well	2×10^5/well	1 µg DNA/well 30 µg/plate	100 pmol in 10 µL tip 200 nM per well	**Buffer R** 10 µL/well 270 µL/plate
Primary Suspension Blood Cells	18-well	$1–2 \times 10^5$/well	0.5–1 µg DNA/well 20 µg/plate	100 pmol in 10 µL tip 200 nM per well	**Buffer T** 10 µL/well 180 µL/plate

3. Set up a Neon® Tube with 3 mL Electrolytic Buffer (use Buffer E for 10 µL Neon® Tip and Buffer E2 for 100 µL Neon® Tip) into the Neon® Pipette Station and Neon® Tip containing the cell-DNA/siRNA mixture.
4. Perform electroporation using the parameters listed on the next page:

Optimization protocol—Day Two, continued

Sample	Well no.	Pulse voltage	Pulse width	Pulse no.	Results	
					Transfection efficiency	Cell viability
1	A1	1450	20	1		
2	A2	1475	20	1		
3	A3	1500	20	1		
4	A4	1525	20	1		
5	A5	1550	20	1		
6	A5	1575	20	1		
7	B1	1375	30	1		
8	B2	1400	30	1		
9	B3	1425	30	1		
10	B4	1450	30	1		
11	B5	1475	30	1		
12	B6	1500	30	1		
13	C1	Control containing DNA but no electroporation pulse.				

5. After electroporation, immediately remove the Neon® Pipette and transfer the samples from the 10 µL Neon® Tip into prewarmed 0.5 mL culture medium.

   For 100 µL Neon® Tip, dilute samples 10-fold in 900 µL medium and transfer 100 µL of the sample to 0.4 mL prewarmed culture medium.
6. Repeat Steps 3–5 for the remaining samples.
7. Gently rock the plate to assure even distribution of the cells. Incubate the plate at 37°C in a humidified CO_2 incubator.
8. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA).
9. Select the best conditions and proceed to the next day's experiment, next page.

Optional: optimization protocol—Day Three

For further optimization, repeat experiments by varying other conditions such as multiple pulsations. **This is optional and depends on the cell type.**

For siRNA, you can vary the amount of siRNA from 10–200 nM.

1. Make sure you have cells prepared as described on pages 16–17, have the DNA or siRNA, and prepare 18- or 24-wells of a 24-well plate containing 0.5 mL culture medium with serum and **without antibiotics** to transfer the electroporated cells.
2. For each electroporation sample using the **10 µL Neon® Tip**, see table below.

   For using the 100 µL Neon® Tip in **24-well** format, adjust the amounts listed in the table below appropriately by 10-fold.

Cell Type	Format	Cell no.	DNA	siRNA	Resuspension Buffer
Adherent	24-well	1×10^5/well	0.5 µg DNA/well 15 µg/plate	50 pmol in 10 µL tip 100 nM per well	**Buffer R** 10 µL/well 285 µL/plate
Suspension	24-well	2×10^5/well	1 µg DNA/well 30 µg/plate	100 pmol in 10 µL tip 200 nM per well	**Buffer R** 10 µL/well 270 µL/plate
Primary Suspension Blood Cells	18-well	$1–2 \times 10^5$/well	0.5–1 µg DNA/well 20 µg/plate	100 pmol in 10 µL tip 200 nM per well	**Buffer T** 10 µL/well 180 µL/plate

3. Set up a Neon® Tube with 3 mL Electrolytic Buffer (use Buffer E for 10 µL Neon® Tip and Buffer E2 for 100 µL Neon® Tip) into the Neon® Pipette Station and Neon® Tip containing the cell-DNA/siRNA mixture.
4. Perform electroporation using the parameters listed on the next page:

***Optional:* Optimization protocol—Day Three**, continued

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

5. After electroporation, immediately remove the Neon® Pipette and transfer the samples from the 10 µL Neon® Tip into prewarmed 0.5 mL culture medium.

   For 100 µL Neon® Tip, dilute samples 10-fold in 900 µL medium and transfer 100 µL of the sample to 0.4 mL prewarmed culture medium.

6. Repeat Steps 3–5 for the remaining samples and incubate the plate.
7. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA).
8. Select the best conditions and save these parameters into the database for your cell type.

Troubleshooting

Problem	Cause	Solution
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.
Connection error message displayed	Pipette or tube is incorrectly inserted	• Properly insert the Neon® Pipette into the Neon® Pipette Station as described on page 20. The metal head of the pipette should be tightly connected to the ball plunger inside the pipette station. • Properly insert the Neon® Tube into the Neon® Pipette Station as described on page 15. The side electrode on the tube should be tightly connected to the ball plunger inside the pipette station. • Avoid spilling any liquid into the pipette station to prevent any build up of rust on the ball plunger in the pipette station.
	The sensor connector is not connected	• Be sure to connect the sensor connector of the Neon® Pipette Station to the sensor port on the rear of the Neon® device. • Make sure the mark on the cable plug and the instrument connector is aligned correctly (page 6)
Error messages	—	See page 33 for a description of error messages.
Connection failure	No Neon® Tip is inserted or the Neon® Tip is inserted incorrectly	Make sure that the Neon® Tip is inserted into Neon® Pipette correctly as described on page 20. 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 3 mL of the appropriate Electrolytic Buffer to Neon® Tube. The electrode in the tube must be completely immersed in buffer. Be sure to add sample in Resuspension Buffer to the Neon® Tip.
	Wrong buffers used	Use the Electrolytic Buffer (Buffer E for 10 µL tip and Buffer E2 for 100 µL tip) in the Neon® Tube and the sample in Resuspension Buffer in the Neon® Tip. Do not switch buffers or use any other buffer as these buffers are specifically designed for electroporation with the Neon® device.
	High voltage connector is not connected	Be sure to connect the high voltage connector of the Neon® Pipette Station to the high voltage port on the rear of the Neon® device (page 6).
If the error persists and all connections are correct	Perform self diagnostics test	Perform self diagnostics test by clicking on ✓ on the main screen. 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 (page 41).

Problem	Cause	Solution
Arcing (sparks)	Air bubbles in the Neon® Tip	Avoid any air bubbles in the Neon® 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 13 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® Tip	**Do not** use the same Neon® Tip for electroporation for more than 2 times because the repeated application of electric pulses reduce the tip quality and impairs their physical integrity.
Low transfection efficiency	Poor optimization of electrical parameters	Perform optimization for your cell type as described on page 22.
	Poor plasmid DNA quality or the plasmid DNA is low	Use high quality plasmid DNA for transfection (see page 13 for guidelines and recommendations on DNA quality). Start with 0.5 µg plasmid DNA per sample.
	Incorrect cell density	Cell densities $>3 \times 10^5$ or $<5 \times 10^4$ 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.
Non-reproducible transfection efficiency	Inconsistent cell confluency or passage number	Always use cells with low passage number and harvest cells with comparable confluency levels.
	Multiple use of Neon® Tip and Neon® Tube	**Do not** use the same Neon® Tip for more than **2 times** because the repeated application of electric pulses reduce the tip quality and impairs their physical integrity. Do not use the same Neon Tube for more than 10 times. Always use a new Neon® Tip and Neon® Tube for different plasmid DNA samples to avoid any cross-contamination.
High energy error	Used high electrical parameters	Set lower voltage or duration.

Neon® Device error messages

Introduction

This section describes the error messages displayed. 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 (page 41) if you need to send the device for servicing.

Error message	Action
Please connect station	The Neon® Pipette Station is not connected properly; ensure that the sensor connector is connected to the sensor port on the rear of the device (page 6).
Check tip for air bubbles.	Remove the solution and aspirate the sample into the tip again without any air bubbles. Press **OK** to exit the screen.
Please enter user name	All protocols in the database need a user name. Enter the user name and press **OK** to exit the screen.
Please enter protocol name	All protocols in the database need a protocol name. Enter the user name and press **OK** to exit the screen.
Password incorrect, please re-enter	Re-enter the 4-digit password and press **OK** to exit the screen.
Input voltage, pulse width, or pulse number error	The input voltage, pulse width, or pulse number is out of range. The valid range is displayed on the screen. Please enter the valid value and press **OK** to exit the screen.

Appendix

Repackaging the instrument

Repackaging and storage instructions

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

Note: Prior to sending the device, ensure the device is properly decontaminated if the device 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 (page 41) for a decontamination protocol and to obtain a Returns Goods Authorization (RGA) number and return shipping instructions.

1. Turn **off** the main power switch at the rear of the device and detach the power cord from the rear of device.
2. Disconnect the high voltage and sensor connector connected to the pipette station via the connector at the back of the unit.
3. Place the instrument in the original box including the original packing foam.
4. Tape the box securely and place appropriate shipping labels for shipping the instrument to Invitrogen. Always transport the box with the unit in the **upright** position.
5. If the device is not to be used for extended periods of time, store the repackaged device in an upright position at 4ºC to 40ºC.

Product specifications

Neon® Transfection System specifications

Operating Power:	100–240 VAC, 2.1 A, 150 W, Frequency 50/60 Hz,
Output:	0.5-2.5 kV
Pulse Width:	1-100 ms
Maximum Duty Cycle:	0.1
Charging Time:	Maximum 8 seconds
Altitude:	Up to 2,000 meters
Operating Temperature:	5ºC to 40°C
Maximum Relative Humidity:	Up to 80%
Degree of Protection:	IPX0
Protective Earthing:	Class I (earthed)
Installation Category:	II
Instrument Type:	Benchtop unit
Device Dimensions:	9.2 inches (w) × 11.8 inches (l) × 8.66 inches (h)
Pipette Station Dimensions:	5.91 inches (diameter); 5.51 inches (h)
Device Weight:	13.2 pounds (6 kg)
Built-in Features:	Touch screen (800 × 480 pixels), digital display

The Neon® Transfection System including the Neon® Pipette Station is compatible with standard nonhazardous laboratory reagents. **Do not** use organic solvents in the tip/tubes or with the device.

Safety information

Safety

Follow the instructions in this section to ensure safe operation of the Neon® Transfection device. The Neon® Transfection System is designed to meet EN61010-1 Safety Standards. To ensure safe, reliable operation, always operate the Neon® Transfection 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 page 35.
- 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	The symbols used on the Neon® Transfection System and in the manual are explained below:
	The **Caution** symbol denotes a risk of safety hazard. Refer to the accompanying documentation.
	ON (power)
	**OFF** (power)
	Protective earth (ground)
	Used on the instrument to indicate an area where a potential shock hazard may exist.
	**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. Visit **www.lifetechnologies.com/weee** for collection and recycling options.
	The CE mark symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the Neon® Transfection 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.

Informations de sécurité

Sécurité

Suivez les instructions de cette section pour vous assurer d'utiliser l'appareil Neon™ Transfection en toute sécurité. Le Neon™ Transfection System est conçu pour répondre aux normes de sécurité EN61010-1. Pour assurer un fonctionnement sûr et fiable, utilisez toujours le Neon™ Transfection 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 35.
- 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.

Symboles d'information	Les symboles utilisés sur le Neon™ Transfection System et dans le manuel sont expliqués ci-dessous :
	Le symbole **Attention** indique un risque d'accident. Consultez la documentation fournie.
	ON (MARCHE) (alimentation)
	**OFF (ARRÊT)** (alimentation)
	Protection par la mise à la terre (masse)
	Utilisée sur l'instrument pour indiquer une zone où existe un risque potentiel d'électrocution.
	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.
	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™ Transfection 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.

Accessory products

Additional products

The following products are for use with the Neon® Transfection System and are available separately.

For more information, visit **www.lifetechnologies.com** or contact Technical Support (page 41).

Product	Quantity	Catalog no.
Neon® Kit, 10 µL	1 kit (50 reactions)	MPK1025
	1 kit (192 reactions)	MPK1096
Neon® Kit, 100 µL	1 kit (50 reactions)	MPK10025
	1 kit (192 reactions)	MPK10096
Neon® Pipette	1 each	MPP100
Neon® Pipette Station	1 each	MPS100
Neon® Tubes	1 pack of 100	MPT100
Dulbecco's Phosphate-Buffered Saline (D-PBS) (1X), liquid without Ca^{2+} and Mg^{2+}	500 mL	14190-144
BLOCK-iT™ Fluorescent Oligo *for electroporation*	75 µL	13750062
Silencer® Select GAPDH Positive Control siRNA (human, mouse, rat)	5 nmol	4390849
Silencer® Select negative Control No. 1 siRNA	40 nmol	4390844
Silencer® Cy™3 labeled GAPDH siRNA (human, mouse, rat)	5 nmol	AM4649
Silencer® FAM labeled GAPDH siRNA (human, mouse, rat)	5 nmol	AM4650
Countess® Automated Cell Counter	1 each	C10227
PureLink™ HiPure Plasmid Miniprep Kit	25 preps	K2100-02
PureLink™ HiPure Plasmid Midiprep Kit	25 preps	K2100-04
PureLink™ HiPure Plasmid Filter Midiprep Kit	25 preps	K2100-14
PureLink™ HiPure Plasmid Maxiprep Kit	25 preps	K2100-07
PureLink™ HiPure Plasmid Filter Maxiprep Kit	25 preps	K2100-17
MagMax™ 96 Total RNA Isolation Kit	96 reactions	AM1830
TaqMan® Gene Expression Cells-to-CT™ Kit	100 reactions	AM1728
alamar Blue®	25 mL	DAL1025

Cell culture media

A large variety of cell culture media and products for mammalian cells including primary and stem cells is available from Invitrogen. For more information, visit **www.lifetechnologies.com** or contact Technical Support (page 41).

siRNA

A large variety of siRNA products including Stealth™ RNAi, *Silencer*® Select RNAi, *Silencer*® RNAi, or standard unmodified siRNA is available from Invitrogen. For more information, visit **www.lifetechnologies.com/rnai** or contact Technical Support (page 41).

Technical support

Obtaining support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com)**
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/sds**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Notes