

Neon™ Transfection System

Catalog no. MPK5000

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Instructions for using the Neon™ Transfection System for transfection of mammalian cells are described below. For detailed instructions, refer to the manual supplied with the product or download the manual from www.invitrogen.com.

General Guidelines

- Prepare high-quality plasmid 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.
- Use an appropriate GFP (green fluorescent protein, Clontech) construct or siRNA control (Invitrogen, Cat. nos. 13750062 or 4390849) to determine transfection efficiency.
- Use Resuspension Buffer R for established adherent and suspension cells as well as primary adherent cells and use **Resuspension Buffer T for primary blood-derived suspension cells**.
- Based on your initial results, you may need to optimize the electroporation parameters for your experiment using an 18-well or pre-programmed 24-well optimization protocol.
- Discard the Neon™ Tips after 2 usages and Neon™ Tubes after 10 usages as a biological hazard. Change the tube and buffer when switching to a different plasmid DNA/siRNA or cell type.
- The volume of plasmid DNA or siRNA added to the transfection reaction should not exceed 10% of the total transfection volume.
- Visit www.invitrogen.com/neon for a library of electroporation protocols for a variety of commonly used cell types.

Preparing Cells

1. Cultivate the required number of cells (see table below for cell amount) such that the cells are 70–90% confluent on the day of the experiment.
2. On the day of the experiment, harvest and wash cells in phosphate buffered saline (PBS) without Ca²⁺ and Mg²⁺.
3. Resuspend the cell pellet in the appropriate Resuspension Buffer (included with Neon™ Kits) at a final density of 1.0 × 10⁷ cells/mL (adherent cells) or 2.0 × 10⁷ cells/mL (suspension cells).
4. Prepare plates by filling the wells with the appropriate volume of culture medium containing serum and supplements **without antibiotics** as described in the table below and pre-incubate plates at 37°C in a humidified 5% CO₂ incubator.

Amount of Reagents

For each electroporation sample, the amount of plasmid DNA/siRNA, cell number, and volume of plating medium **per well** are listed in the table below. **Be sure to use Resuspension Buffer T for primary suspension blood cells and Resuspension Buffer R of all other cell types.**

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

Intended Use: For research use only. Not intended for any animal or human therapeutic or diagnostic use

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Using the Neon™ Transfection System

Refer to the manual for details on setting up the Neon™ device and Neon™ Pipette Station.

There are 3 options to select an electroporation protocol for your cell type:

- Input the electroporation parameters in the Input window, if you already have the electroporation parameters for your cell type.
 - Press **Database** button on the touchscreen and select cell-specific electroporation parameters that you have added for various cell types.
 - Press **Optimization** button on the touchscreen to perform the optimization protocol for your cell type.
1. Fill the Neon™ Tube with 3 mL Electrolytic Buffer (use Buffer E for 10 µL Neon™ Tip and Buffer E2 for 100 µL Neon™ Tip).
 2. Insert the Neon™ Tube into the Neon™ Pipette Station until you hear a click sound (Figure 1).
 3. Transfer the appropriate amount of plasmid DNA/siRNA into a sterile, 1.5 mL microcentrifuge tube.
 4. Add cells to the tube containing plasmid DNA/siRNA and gently mix. See the table on back side for cell number, DNA/siRNA amount, and plating volumes to use.
 5. Press the push-button on the Neon™ Pipette to the second stop to open the clamp and insert the top-head of the Neon™ Pipette into the Neon™ Tip until the clamp fully picks up the mounting stem of the piston. 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 (Figure 2A and B).
 6. 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 (Figure 3).
- Note:** Avoid air bubbles during pipetting as air bubbles cause arcing during electroporation. If you notice air bubbles in the tip, discard the sample and carefully aspirate fresh sample into the tip again without any air bubbles.
7. Insert the Neon™ Pipette with the sample vertically into the Neon™ Tube placed in the Neon™ Pipette Station until you hear a click sound (Figure 4).
 8. Ensure that you have selected the appropriate electroporation protocol and press **Start** on the touchscreen (Figure 5).
 9. The Neon™ device automatically checks for the proper insertion of the Neon™ Tube and Neon™ Pipette before delivering the electric pulse.
 10. The touchscreen displays **Complete** to indicate that the electroporation is complete.
 11. 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 prewarm medium with serum and supplements but **without antibiotics** (Figure 6).
- Discard the Neon™ Tip into an appropriate biological hazardous waste container.
12. Repeat Steps 5–11 for the remaining samples.
- 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/siRNA or cell type.
13. Gently rock the plate to assure even distribution of the cells. Incubate the plate at 37°C in a humidified CO₂ incubator.
 14. If you are not using the Neon™ device, turn the power switch on the rear to **OFF**.
 15. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA).
 16. Based on your initial results, you may need to optimize the electroporation parameters for your cell type. Refer to the manual for using the 18-well or preprogrammed, 24-well optimization protocol on the Neon™ device.

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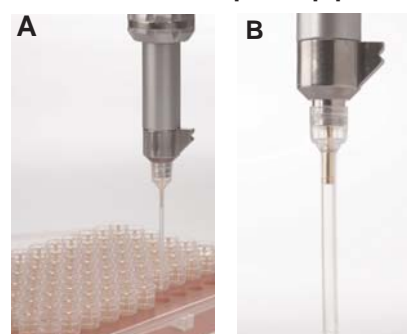
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1. Insert Neon™ Tube with buffer



2. Insert Neon™ Tip into pipette



3. Aspirate sample into Neon™ Pipette



4. Insert Neon™ Pipette



5. Press Start



6. Transfer samples to medium



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