Neon™ Transfection System

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

Catalog Numbers MPK5000, MPK1025, MPK1096, MPK10025, MPK10096

Doc. Part No. 25-1056 Pub. No. MAN0001632 Rev. B.0

WARNING! For safety and biohazard guidelines, see the "Safety" appendix in the *Neon™ Transfection System User Guide* (Pub. No. MAN0001557). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Note: This Quick Reference is intended as a benchtop reference for experienced users of the *Neon™ Transfection System User Guide* (Pub. No. MAN0001557). For detailed instructions, supplemental procedures, and troubleshooting, see the *Neon™ Transfection System User Guide* (Pub. No. MAN0001557).

General guidelines

- Prepare high-quality plasmid DNA at a concentration of 1 to 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) construct or siRNA control to determine transfection efficiency. See the *Neon™ Transfection System User Guide* (Pub. No. MAN0001557) for details.
- Use Resuspension Buffer R for established adherent and suspension cells, as well as primary adherent cells. Use Resuspension Buffer T with high voltage protocols of 1900 V or more. If arcing occurs with Resuspension Buffer R, consider switching to Resuspension Buffer T.
- 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 tranfection reaction should not exceed 10% of the total transfection volume.
- Visit **thermofisher.com** for a library of electroporation protocols for a variety of commonly used cell types.

Prepare cells

For the appropriate volume of medium to use based on cell density, or plating volumes for other plate formats, see "Amount of reagents" on page 2.

1. Cultivate the required number of cells (70% to 90% confluent on the day of transfection) by seeding a flask containing fresh growth medium 1 to 2 days prior to eletroporation.
2. On the day of the experiment, pre-warm aliquots of culture medium containing serum, PBS (without $Ca^{2+}$and Mg^{2+}), and Trypsin/EDTA solution to 37°C.
3. Rinse the cells with PBS (without $Ca^{2+}$and Mg^{2+}), then trypsinize the cells with the Trypsin/EDTA solution.
4. Take an aliquot of trypsinized cell suspension, then count cells to determine the cell density.
5. Harvest the cells in growth medium containing serum.
6. Transfer cells to a 1.5-mL microcentrifuge tube or a 15-mL conical tube, then centrifuge the cells at 100 - 400 × *g* for 5 minutes at room temperature.
7. Wash cells with PBS (without $Ca^{2+}$and Mg^{2+}) by centrifugation at 100 - 400 × *g* for 5 minutes at room temperature.
8. Aspirate the PBS, then resupsend the cell pellet in Resuspension Buffer R (or Resuspension Buffer T for programs ≥ 1900 V) at a final density of 1.0×10^7 cells/mL for adherent cells or 2.0×10^7 cells/ml for suspension cells. Gently pipette the cells to obtain a single cell suspension.

   IMPORTANT! Avoid storing the cell suspension for more than 15 to 30 minutes at room temperature. This will reduce cell viability and transfection efficiency.
9. Prepare 24-well plates by filling the wells with 500 µL of culture medium containing serum and supplements, but without antibiotics. Pre-incubate plates in a 37°C, 5% CO^2 humidified 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 following table. Use Resuspension Buffer T for cell types that require high voltage protocols of 1900 V or more. For all other cell types, use Resuspension Buffer R.

Format	Cell Type	DNA (µg)	siRNA (nM)	Neon™ Tip	Volume of plating medium	Cell Number	Buffer R or T[1]
96-well	Adherent	0.25–0.5	10–200	10 µL	100 µL	1–2 × 10^4	10 µL/well
	Suspension	0.5–1		10 µL		2–5 × 10^4	10 µL/well
48-well	Adherent	0.25–1	10-200	10 µL	250 µL	2.5–5 × 10^4	10 µL/well
	Suspension	0.5–2		10 µL		5–12.5 × 10^4	10 µL/well
24-well	Adherent	0.5–2	10-200	10 µL	500 µL	0.5–1 × 10^5	10 µL/well
	Suspension	0.5–3		10 µL		1–2.5 × 10^5	10 µL/well
12-well	Adherent	0.5–3	10-200	10 µL	1 mL	1–2 × 10^5	10 µL/well
	Suspension	0.5–3		10 µL		2–5 × 10^5	10 µL/well
6-well	Adherent	0.5–3 (10 µL) 5–30 (100 µL)	10-200	10 µL/100 µL	2 mL	2–4 × 10^5	10 µL or 100 µL/well
	Suspension	0.5–3 (10 µL) 5–30 (100 µL)		10 µL/100 µL		0.4–1 × 10^6	10 µL or 100 µL/well
60 mm	Adherent	5–30	10-200	100 µL	5 mL	0.5–1 × 10^6	100 µL/well
	Suspension	5–30		100 µL		1–2.5 × 10^6	100 µL/well
10 cm	Adherent	5–30	10-200	100 µL	10 mL	1–2 × 10^6	100 µL/well
	Suspension	5–30		100 µL		2–5 × 10^6	100 µL/well

[1] **Use Resuspension Buffer T for primary suspension blood cells.**

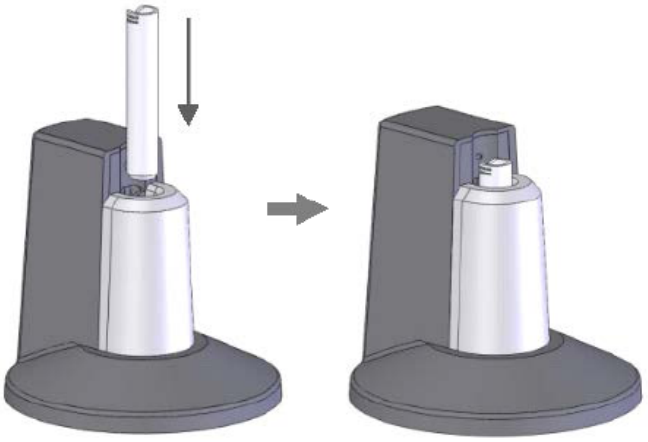
Using the Neon™ Transfection System

For details on setting up the Neon™ device and Neon™ Pipette Station, see the *Neon™ Transfection System User Guide* (Pub. No. MAN0001557).

1. Select the appropriate protocol for your cell type. Use one of the following options:
   - Input the electroporation parameters in the **Input** window if you already have the electroporation parameters for your cell type.
   - Tap **Database**, then select the cell-specific electroporation parameters that you have added for various cell types.
   - Tap **Optimization** to perform the optimization protocol for your cell type.
2. Fill the Neon™ Tube with 3 mL of Electrolytic Buffer (use Buffer E for the 10 µL Neon™ Tip and Buffer E2 for the 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 (Figure 1).

Figure 1 Schematic of Neon™ Tube and Neon™ Pipette Station.



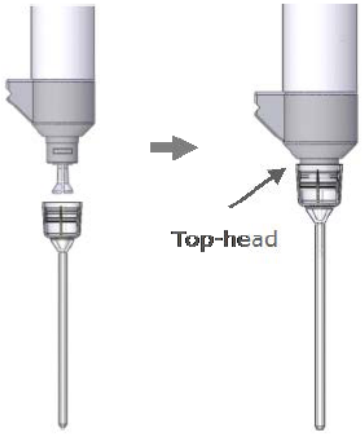
4. Transfer the appropriate amount of plasmid DNA/siRNA into a sterile, 1.5 mL microcentrifuge tube.

5. Add cells to the tube containing plasmid DNA/siRNA, then gently mix. See “Amount of reagents” on page 2 for cell number, DNA/siRNA amount, and plating volumes to use.

6. To insert a Neon™ Tip into the Neon™ Pipette, press the push-button on the pipette to the second stop to open the clamp.

7. Insert the top-head of the Neon™ Pipette into the Neon™ Tip until the clamp fully picks up the mount stem of the piston (Figure 2).

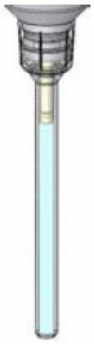
Figure 2 Schematic of Neon™ Pipette and Neon™ Tip.



8. 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.

9. 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).

Figure 3 Schematic of 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, then carefully aspirate the fresh sample into the tip again without any air bubbles.

10. 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).

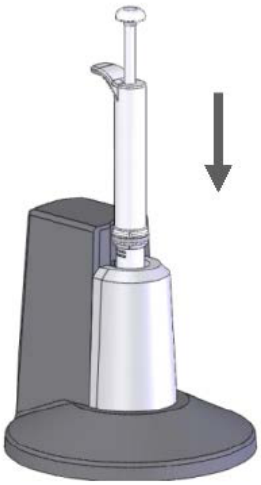


Figure 4 Schematic of Neon™ Tube and Neon™ Pipette Station.

Note: Ensure that the metal head of the Neon™ pipette projection is inserted into the groove of the pipette station.

11. Ensure that you have selected the appropriate electroporation protocol, then press **Start** on the touchscreen.

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

13. After delivering the electric pulse, **Complete** is displayed on the touchscreen to indicate that electroporation is complete.

14. Slowly remove the Neon™ Pipette from the Neon™ Pipette Station. 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 with serum and supplements but without antibiotics.

Note: Discard the Neon™ Tip into an appropriate biological hazardous waste container. To discard the Neon™ Tip, press the push-button to the second stop into an appropriate biological hazardous waste container.

15. Repeat step 6 to step 14 for the remaining samples.

Note: 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.

16. Gently rock the plate to ensure even distribution of the cells. Incubate the plate at 37°C in a humidified CO_2 incubator.

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

18. Assay samples to determine the transfection efficiency (e.g., fluorescence microscopy or functional assay) or gene knockdown (for siRNA).

19. Based on your initial results, you may need to optimized the electroporation parameters for your cell type. For more information, see the *Neon™ Transfection System User Guide* (Pub. No. MAN0001557).

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