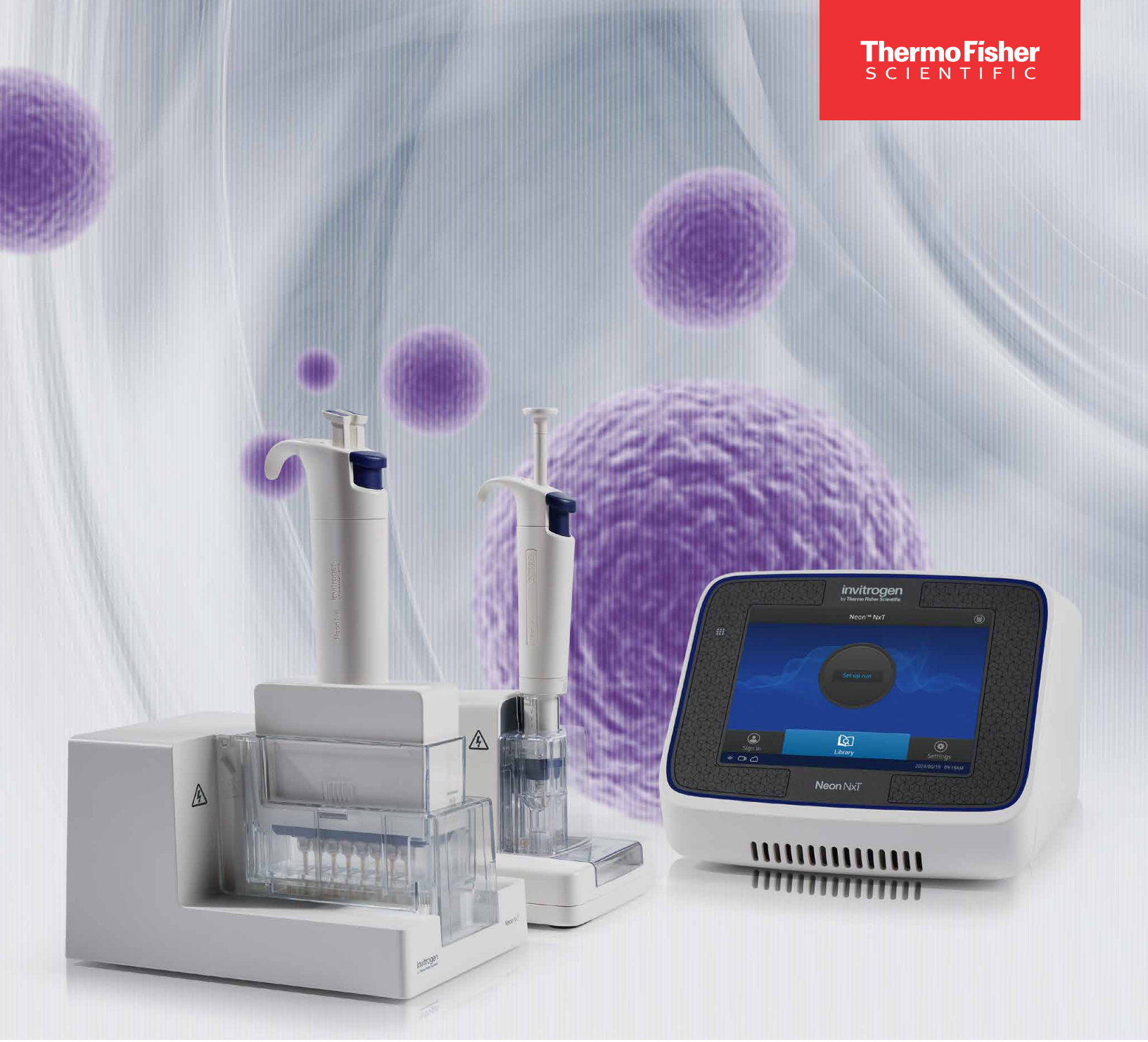
ThermoFisher SCIENTIFIC



Electroporation

Transfect with the best of what's NxT

Neon NxT Electroporation System

invitrogen

Introducing the Neon NxT Electroporation System

The Invitrogen™ Neon™ NxT Electroporation System is a next-generation electroporation platform with an innovative design that streamlines the mammalian cell transfection workflow. The electrodes in the biologically compatible pipette tip and buffer tube generate a more uniform electric field than traditional electroporation devices, significantly increasing transfection efficiency and post-transfection cell viability.

The Neon NxT pipette tip serves as the transfection chamber, so there is no need to transfer your sample to a separate electroporation cuvette. Reducing the number of pipetting steps helps save time, minimize sample loss, reduce the amount of shear force experienced by cells, and mitigate the risk of sample contamination. The surprisingly simple Neon NxT electroporation workflow can help transfect even your most challenging cell lines with confidence.

Benefits of the Neon NxT Electroporation System

Proven performance and exceptional cell viability—Confidently transfect challenging cell lines and preserve cell viability with our proprietary electroporation tip technology.

Sample preservation—The unique Neon NxT pipette tip eliminates the need for a separate electroporation cuvette or plate, while the compact design of the instrument enables an easy fit within the biosafety cabinet. Your valuable cells are not lost through sample transfer and are at reduced risk of contamination.

Time-saving—Transfect cells in 3 easy steps with a single buffer kit. Simply aspirate your cells and delivery payload into the Neon NxT pipette tip, place it in the pipette station, and select the electroporate option. Your transfected cells are now ready for transfer to a culture vessel. With the Invitrogen™ Neon™ NxT 8-Channel Pipette and plate format, you can electroporate 96 samples in less than 15 minutes.

Flexibility—Precisely optimize your electroporation parameters for different cell types, cell densities, payloads, and applications. Transfect anywhere from 1 x 10^4 to 1 x 10^7 cells per electroporation.

Transfect with the best of what's NxT thermofisher.com/neonnxt

Contents

Proven transfection efficiency

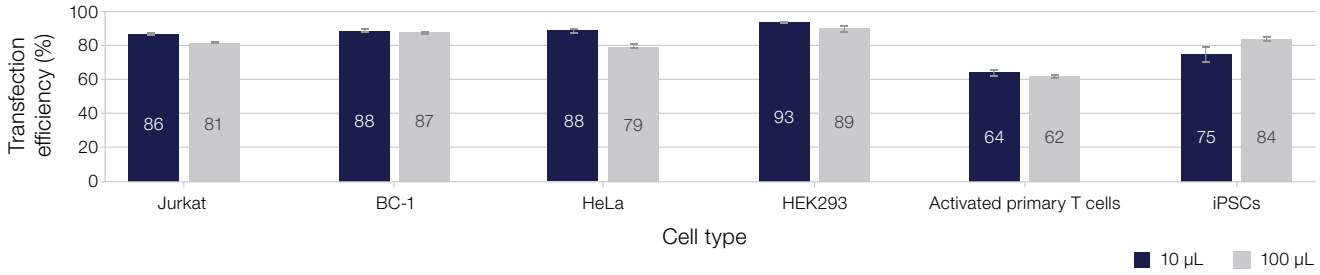
With our proprietary electroporation tip technology, you can efficiently transfect challenging mammalian cells while maintaining high cell viability. We have observed exceptional transfection efficiency and cell viability (Figure 1), even with extremely difficult cells like immune, primary, and stem cells.

Exceptional post-transfection cell viability

We understand how important your cells are. The Neon NxT Electroporation System enables electroporation under more controlled conditions and eliminates two pipetting steps compared to traditional electroporation systems. Cells also experience less shear force, since they do not need to be pipetted into and out of a separate cuvette for electroporation. These features help minimize cell mortality, which is especially important when working with cells that are difficult to grow or transfect (Figure 1).

The electrodes in the Neon NxT Electroporation System are spaced widely apart and have a minimal surface area. The disposable Neon NxT pipette tip houses one of the electrodes, while the other is located at the bottom of the Neon NxT buffer tube. The small surface area creates a more uniform electric field with minimal changes in pH. Less heat and fewer ions are generated during electroporation, so physiological conditions are not severely disturbed.

Transfection efficiency



Viability

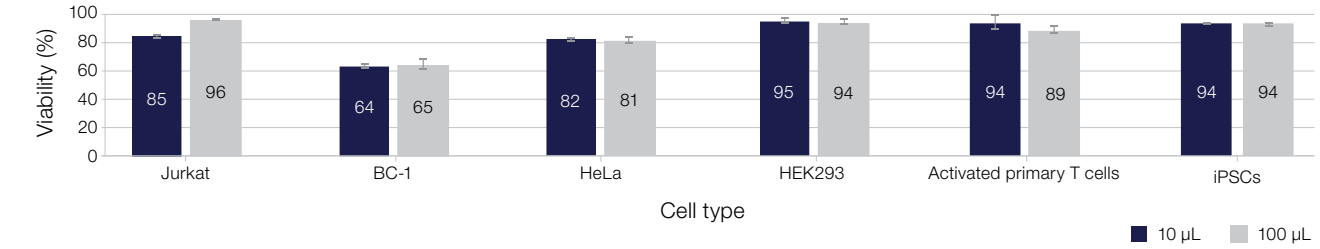


Figure 1. Exceptional transfection efficiency and post-transfection cell viability across diverse cell types are obtained with the Neon NxT Electroporation System. Cells were transfected with GFP plasmid DNA in 10 µL or 100 µL electroporation reactions. Transfection efficiency is reported as the percentage of GFP-positive cells (n = 3). Transfected cells were stained with Invitrogen™ SYTOX™ Red Dead Cell Stain and assessed for viability on the Invitrogen™ Attune™ NxT Flow Cytometer. Cell viability is reported as the mean of 3 measurements.

Did you know?

Our electroporation tip technology has been cited in **more than 14,000 publications**.

The Neon NxT system enables high cell viability by:

- Creating a more uniform electric field
- Maintaining a stable pH throughout the electroporation chamber
- Generating minimal heat
- Forming fewer ions
- Reducing shear force experienced by cells

The biologically compatible Neon NxT pipette tip (left) is designed with proven electroporation tip technology that has important advantages over conventional electroporation technology.

Did you know?

The Neon NxT Electroporation System has an **energy calculator** that helps prevent arcing and delivery of overly energetic pulses. It also has a **built-in arc detection** feature that can enable you to identify unsuccessfully processed samples.

Preserve your precious samples

Minimal sample transfer loss

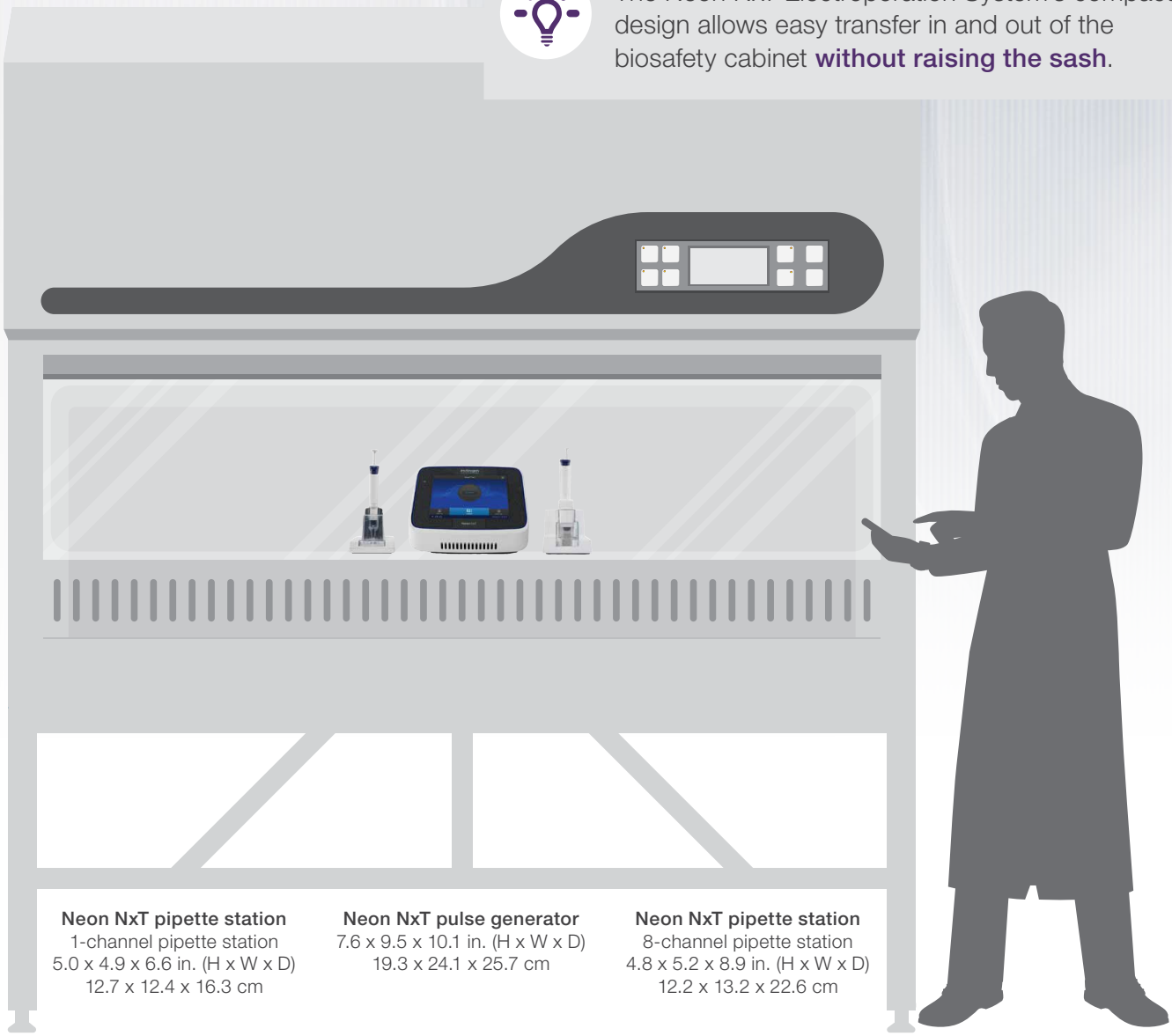
Your samples are precious, but some volume is inevitably lost during transfer to and from a conventional cuvette. Electroporation on the Neon NxT Electroporation System occurs within the pipette tip, not a cuvette, helping minimize sample loss and the amount of shear force experienced by cells.

Minimal sample contamination

The compact footprint of the Neon NxT system, which includes the pulse generator and pipette station, makes it small enough to fit in most biosafety cabinets (BSCs). This helps ensure your samples stay within the aseptic area at all times while using the instrument, helping reduce the risk of contaminating precious cells. The Neon NxT system also has a cable management feature that makes using it in the BSC hassle-free.

Did you know?

The Neon NxT Electroporation System's compact design allows easy transfer in and out of the biosafety cabinet **without raising the sash**.



Neon NxT pipette station
1-channel pipette station
5.0 x 4.9 x 6.6 in. (H x W x D)
12.7 x 12.4 x 16.3 cm

Neon NxT pulse generator
7.6 x 9.5 x 10.1 in. (H x W x D)
19.3 x 24.1 x 25.7 cm

Neon NxT pipette station
8-channel pipette station
4.8 x 5.2 x 8.9 in. (H x W x D)
12.2 x 13.2 x 22.6 cm

Enhance genome editing efficiency

Genome editing holds great promise in the pursuit of a deeper understanding of human health and disease, including helping to transform the drug discovery process, the generation of disease models, and the development of cell and gene therapies.

A critical yet challenging step in the genome editing workflow is the effective delivery of CRISPR ribonucleoprotein (RNP), DNA, and RNA molecules into the cell line of choice—also known as transfection. There are many methods of delivery; however, electroporation is the most widely used method due to its ability to achieve high transfection efficiency, even with hard-to-transfect cells.

The Invitrogen™ Neon™ NxT Resuspension Genome Editing Buffer was specifically designed to increase genome editing efficiency by driving homology-directed repair (HDR) after transfection. When used with the Neon NxT Electroporation System and our gene editing reagents, the Neon NxT Resuspension Genome Editing Buffer can help improve gene editing performance with specific payloads, like CRISPR-Cas9, especially when it comes to knock-in experiments with mammalian cells, including primary, stem, and difficult-to-transfect cells (Figure 2). For additional data, please see our **application note** on achieving exceptional genome editing efficiency with difficult cell types.

Find out more about our comprehensive suite of gene editing workflow solutions at **thermofisher.com/geneediting**.

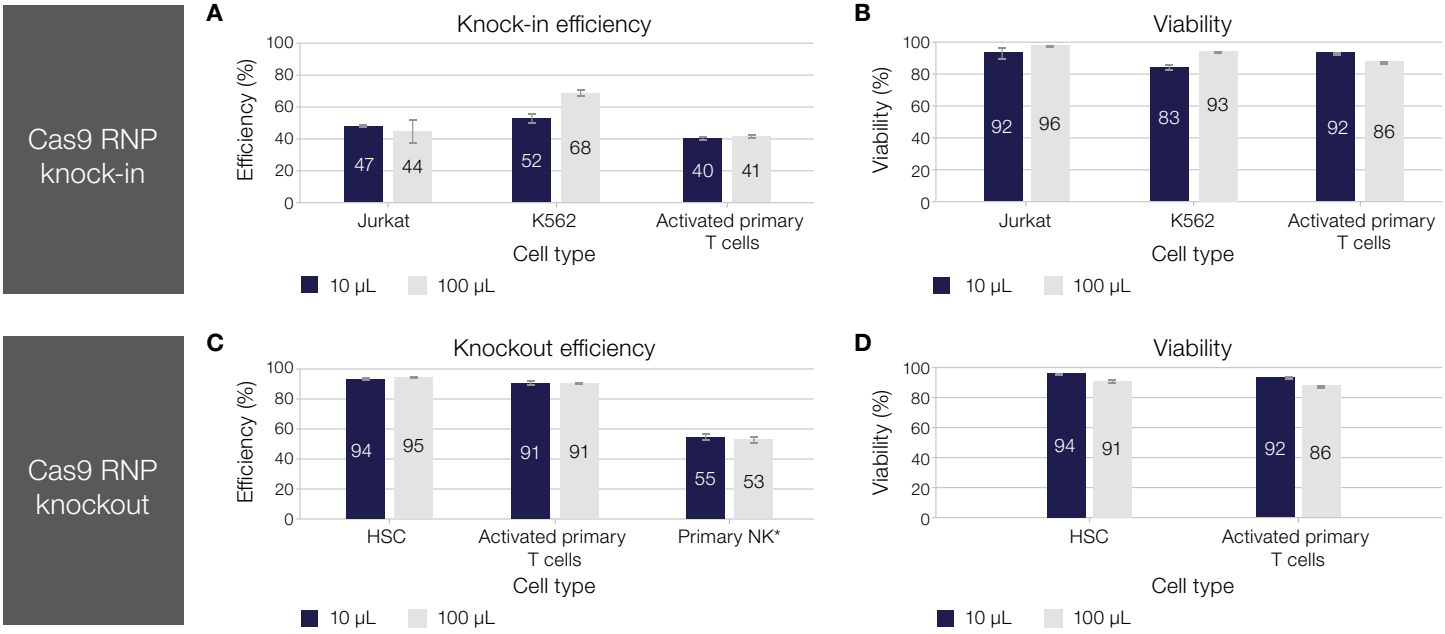


Figure 2. Performance of Neon NxT Resuspension Genome Editing Buffer in genome editing experiments based on CRISPR-Cas9 across diverse cell types and targets. The target loci included *ACTN* for Jurkat and K562 cells, *TRAC* for activated primary T cells, *B2M* for HSCs, and *AAVS1* for primary NK cells. Cells were electroporated in 10 µL or 100 µL reactions. **(A)** GFP donor DNA knock-in efficiency reported as the percentage of GFP-positive cells. **(B)** Viability of cells after GFP donor DNA knock-in. **(C)** Knockout efficiency reported as the percent reduction of a specified target locus compared to untreated control. **(D)** Post-electroporation viability of knockout cells.

* For primary NK cells, indel efficiency (%), determined through a genomic cleavage detection (GCD) assay, served as a proxy for knockout efficiency.

Did you know?

Neon NxT protocols for gene editing have been optimized using **Invitrogen™ TrueGuide™ Synthetic gRNAs** and **Invitrogen™ TrueCut™ Cas9 proteins**. TrueCut Cas9 proteins are also available for both clinical and translational applications, supporting your needs every step of the way.

Flexibility when you need it

Customizable parameters

You need a simple, straightforward workflow with minimal processing time. You also want the ability to quickly fine-tune specific parameters to optimize your process. With the Neon NxT Electroporation System, you can precisely control the parameters that matter most for your experiment without wasting precious time adjusting those that don't. You can modify:

- Pulse voltage
- Pulse width
- Number of pulses
- Cell type
- Buffer type
- Payload type

Versatility for different payloads, cell types, and cell densities

Deliver DNA, RNA, or protein to a wide variety of mammalian cells, with the flexibility to transfect 1 x 10^4 to 1 x 10^7 cells per reaction. We have over 150 **electroporation protocols** and a citation library, which can be filtered by cell line, cell type, payload, product, and type of document (protocol, app note, publication), for a variety of transfection applications.

One buffer kit for many mammalian cell types

Avoid the hassle of searching for a buffer kit that will work with your cell line. We have simplified the process with one buffer kit that is compatible with over 150 mammalian cell lines.

- Invitrogen™ Neon™ NxT Resuspension R Buffer (R buffer) is optimized for efficient and gentle electroporation of mammalian cells and is excellent for transfecting a wide range of cell types, including primary cells and stem cells.
- The Invitrogen™ Neon™ NxT Resuspension T Buffer (T buffer) is designed for hard-to-transfect cell types, specifically for those that require high voltage (>1,900 V). This buffer is formulated to provide high transfection efficiency while maintaining high cell viability.
- Neon NxT Electroporation kits include the Invitrogen™ Neon™ NxT Resuspension Genome Editing Buffer (GE buffer), which greatly enhances genome editing efficiency when combined with the Neon NxT Electroporation System and the Gibco™ CTS™ TrueCut™ Cas9 Protein v2.

Did you know?

The Neon NxT Electroporation System can store **up to 10,000 protocols** for future experiments.

Did you know?

The Invitrogen™ **TransfectionSelect™ tool filters the protocol library** based on the criteria you enter, so you can find the most appropriate protocol for your application.

Did you know?

The Neon NxT Resuspension Genome Editing Buffer can improve **knock-in efficiency up to 5-fold** compared to R buffer.

Save your research time

Simplified workflow

When working with instruments, we need something that is easy for everyone to use, whether they're a beginner or an expert. The Neon NxT system is operated with 3 simple steps: aspirate, electroporate, and dispense. Easy to follow and execute, the streamlined workflow requires minimal training and helps improve consistency and reproducibility.

Shorter processing time

In addition to simplifying the workflow, our proprietary electroporation technology shortens the overall process to 10–15 minutes, compared to a conventional electroporation system [1]. No need to cap, uncap, and pipette samples into and out of a separate electroporation cuvette—transfection occurs in the Neon NxT pipette tip.

Neon NxT electroporation workflow

Step 1

Prepare cells
Prepare cells by suspending them in a Neon NxT buffer.

Step 2

Electroporate
Apply electrical pulses to cells to deliver the payload in the specialized buffer.

Step 3

Return cells to growth conditions
Restore growth conditions to allow the cells to recover.

Step 4

Analyze cells
Evaluate: e.g., gene expression, genome editing, silencing, and cell line development

Did you know?

With **Thermo Scientific™ ClipTip™ technology**, the Neon NxT pipette tip will click when it is in place. The low forces involved in tip attachment and ejection also provide an **ergonomic benefit**.

Time-saving plate setup

The intuitive user interface of the Neon NxT Electroporation System provides an efficient way to set up your electroporation experiments. Instead of adjusting electroporation parameters between samples with Quick Run, you can set up an entire plate beforehand and monitor progress on the screen as you electroporate each of your samples.

Did you know?

Using the Neon NxT 8-Channel Pipette, you can electroporate **up to 96 samples** in less than **15 minutes**.

Plan with ease using the TransfectionLab cloud-based app

You can optimize your electroporation parameters to help increase productivity and improve traceability using the Invitrogen™ TransfectionLab™ cloud-based app found on the **Thermo Fisher™ Connect Platform**. After you enter your experiment details, a step-by-step guide will be generated, tailored to your experiment from the design stage.

Did you know?

You can **remotely set up** multiple plate layouts for up to 384 samples, and any protocol or plate layout saved in the app can be accessed through the cloud rather than the user interface of the Neon NxT system.

8-Channel pipette, greater throughput maintaining performance

With the Neon NxT 8-Channel Pipette, we elevate flexibility to an unprecedented level. Each tip of the 8-channel pipette operates independently within its own isolated tube, enabling the use of different electroporation parameters, resuspension buffers, delivery payloads, or even cell types for each tip. Additionally, the Neon NxT 8-Channel Pipette and Pipette Station work with the existing Neon NxT device as well as leverage the same Neon NxT tips and buffers. Whether you are optimizing conditions or exploring multiple variables, the Neon NxT 8-Channel Pipette provides the versatility needed to help accelerate your research and achieve reliable results.

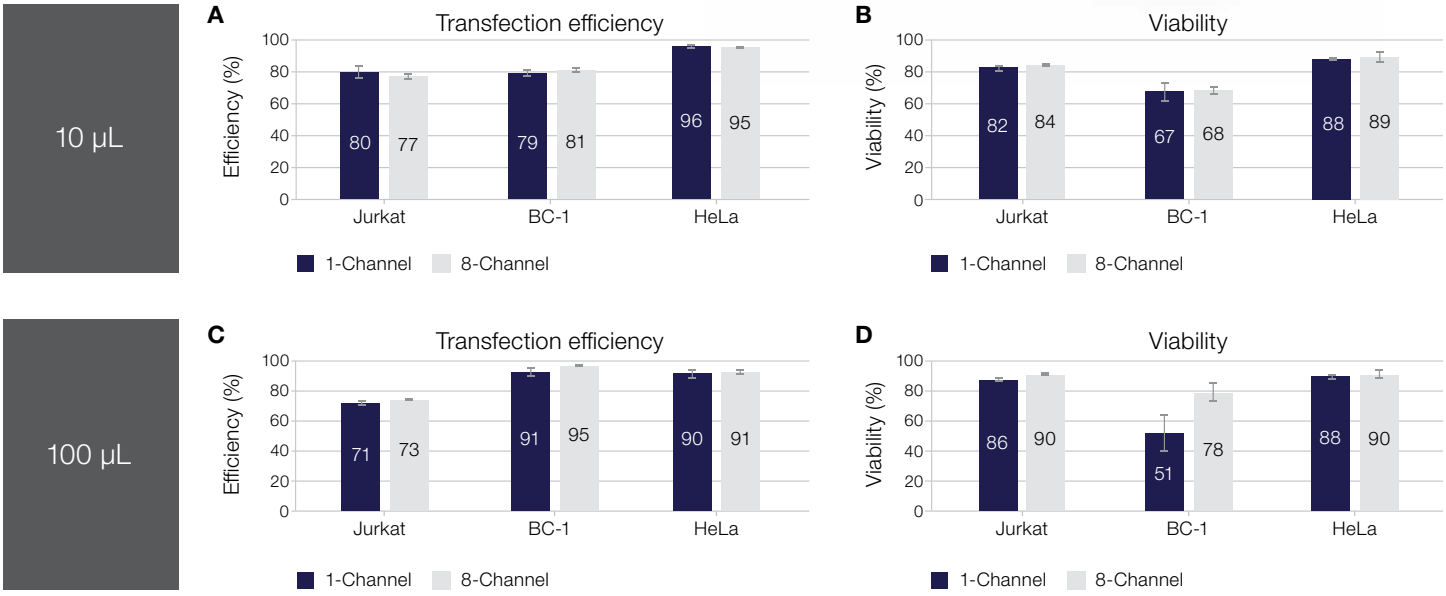


Figure 3. Neon NxT 1-channel vs. 8-channel pipette performance comparison for different cell types. (A) Transfection efficiency (% GFP^+ cells) in 10 µL reaction. **(B)** Transfection viability in 10 µL reaction. **(C)** Transfection efficiency (% GFP^+ cells) in 100 µL reaction. **(D)** Transfection viability in 100 µL reaction. For all the bar graphs n = 8.

Flexibility to maximize optimization

With the Neon NxT 8-Channel Pipette where each channel can be a different condition, you can efficiently test a wide range of parameters in a single experiment, greatly enhancing your research throughput and precision. In Figure 4, we evaluated the optimal electroporation program and resuspension buffer for 11.5 kb GFP plasmid transfection in Jurkat cells. Each data point, representing a specific electroporation program, shows the percentage of GFP$^+$ cells 24 hours post-electroporation against the total number of viable transfected cells (TVTC). The red circles highlight the best-performing conditions, achieving up to 85% transfection efficiency with T buffer. This streamlined approach allows for rapid and accurate optimization, helping to ensure high efficiency and viability in your experiments.

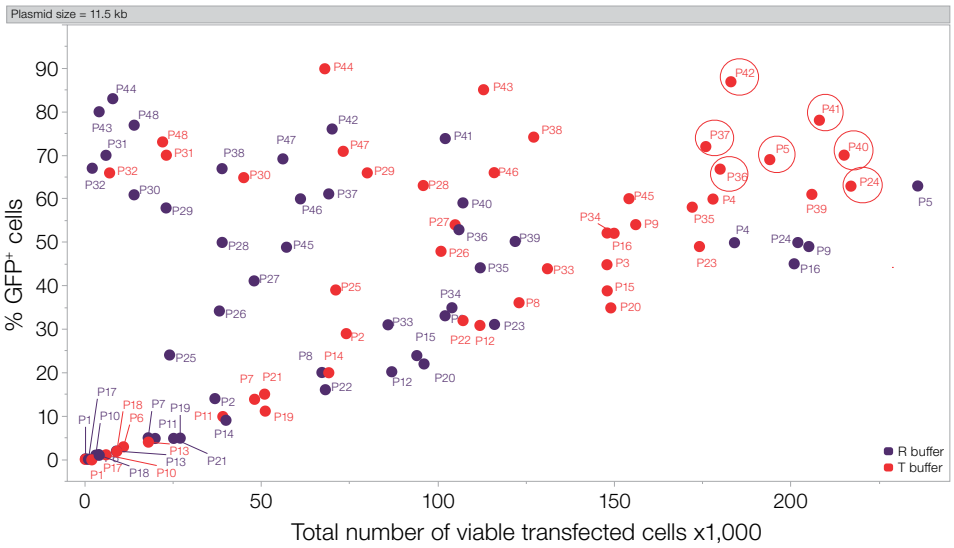


Figure 4. Resuspension R vs. T buffer electroporation performance with 48 programs for 11.5 kb GFP plasmid. % GFP$^+$ cells was plotted against TVTC. Each data point (n = 1) in the graph represents a specific electroporation program. The red circles in the graph indicate the data points that represent the best-performing conditions, characterized by a delicate balance between higher percentages of GFP$^+$ cells and higher TVTCs.

Enhance performance with buffer optimization

Selecting the right buffer for your specific application is crucial for achieving optimal transfection efficiency and viability. While each resuspension buffer is generally recommended for different purposes, true optimization helps ensure you've chosen the best one. As illustrated in Figure 5, CRISPR RNP delivery into activated primary T cells performs best with the GE buffer. Conversely, for Jurkat cells, the R buffer is more suitable for smaller plasmids, whereas the T buffer excels with larger plasmids. Tailoring your buffer choice through careful optimization can significantly enhance your experimental outcomes.

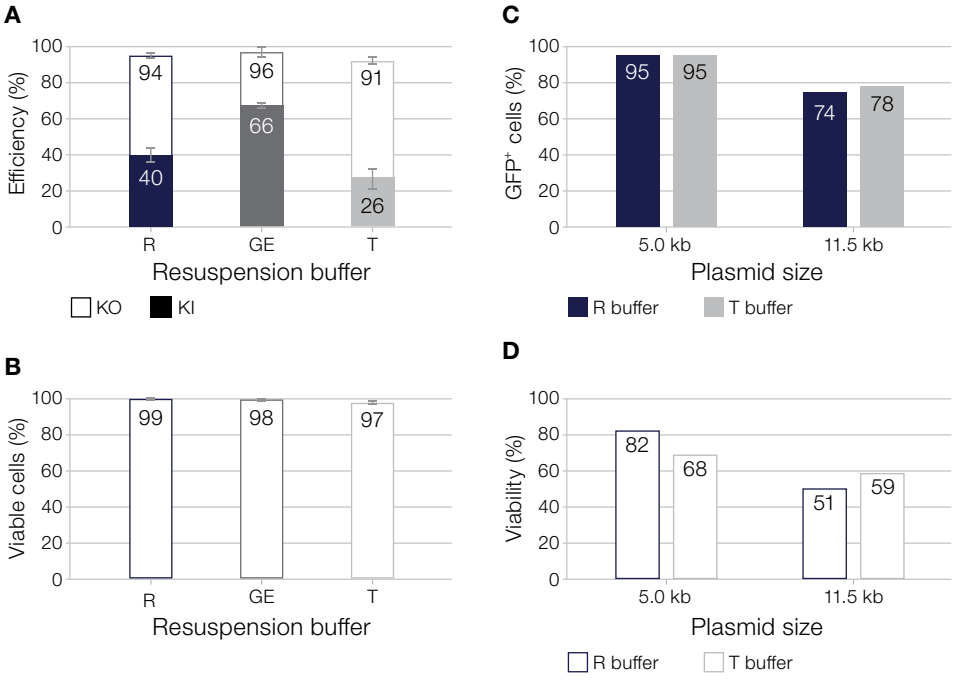


Figure 5. Evaluation of Neon NxT resuspension buffers across various applications. **(A)** Comparison of editing efficiency using resuspension R, GE, and T buffers for CRISPR/Cas9-mediated knockout/knock-in (KO/KI) applications in activated primary T cells. **(B)** Corresponding cell viability for resuspension R, GE, and T buffers. **(C)** Assessment of transfection efficiency using resuspension R and T buffers for delivering plasmids of varying sizes into Jurkat cells. **(D)** Corresponding cell viability for resuspension R and T buffers.

Accelerating the optimization process further

Compatible with design of experiments (DOE) methodologies, the Neon NxT system with the 8-channel pipette empowers researchers to simultaneously explore and optimize multiple variables, enhancing the reliability and robustness of results. By utilizing DOE, you can identify interdependencies among various factors and predict optimal conditions that may not have been directly tested, enabling comprehensive and efficient optimization. This system is exceptional for DOE-based biological studies, allowing for rapid and effective parameter screening.

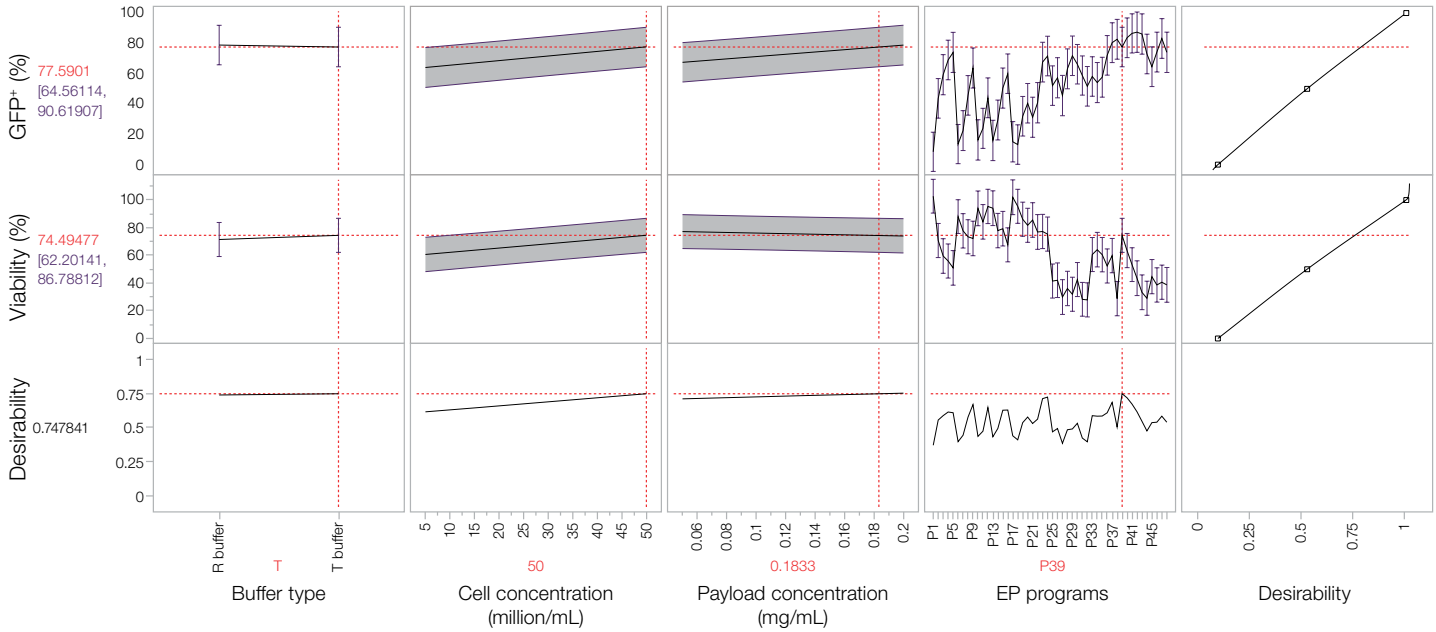


Figure 6. DOE predictive plot utilizing JMP software. The input factors are displayed on the x-axis, while the y-axis represents the predicted responses. The vertical red lines indicate the current value of each factor. The current values of the factors are highlighted in red below the horizontal axis. The red values on the vertical axis represent the predicted responses based on the current values of the factors. These current values were generated by the predictive model as the most optimal values to achieve maximum desirability for each response with equal importance.

Did you know?

We have **installation qualification and operation qualification (IQOQ) available** as an option executed by a field service engineer for the Neon NxT system, to help save you time and effort.

Frequently asked questions

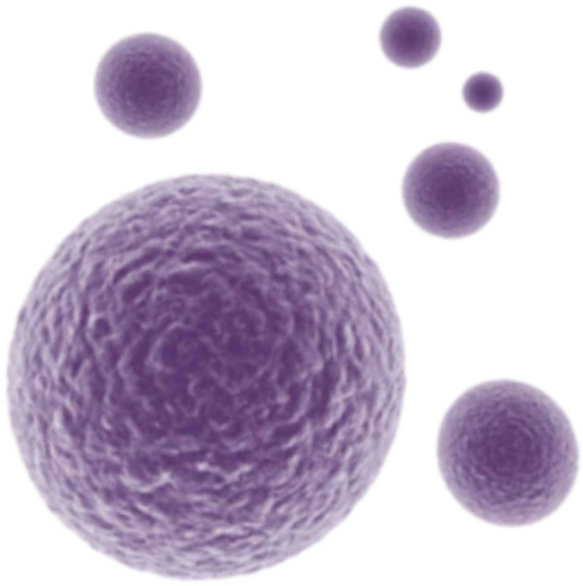
Q: How does the Neon NxT Electroporation System differ from the Invitrogen™ Neon™ Transfection System?
A: The Neon NxT Electroporation System relies on the same unique and trusted electroporation technology, but it has features that make it easier to use, as well as a buffer designed for genome editing applications and a multichannel pipette for up to 8 reactions at once. The Neon NxT pulse generator has an improved feedback loop with user interface notification, and ClipTip technology has been incorporated into Neon NxT pipette tips for secure attachment and easy ejection, along with other ergonomic design improvements.

We transfected several mammalian cell lines with GFP plasmid DNA or GFP mRNA on both systems. Transfection efficiency and post-electroporation cell viability are compared in Figure 3.

Q: What are the advantages of the Neon NxT electrode design?
A: The gold-coated electrodes produce a more uniform electrical field and a lower pH gradient across cell suspensions. The unique design helps maintain physiological conditions, so cells have much higher survival rates after electroporation than cells subjected to conventional electroporation [1].

Q: How do you calibrate the Neon NxT pipettes?
A: The Neon NxT pipettes are permanently calibrated by the manufacturer, so you do not need to calibrate them yourself.

Q: How many times can I use a Neon NxT pipette tip?
A: To avoid carry-over contamination from one sample to another, we recommend using a Neon NxT pipette tip no more than twice. Reusing pipette tips can also lead to oxide formation on the piston surface, which will interfere with electrode function.



Q: What are the differences between the resuspension buffers in the kit, and do any of them contain components with animal origins?
A: Neon NxT Resuspension R Buffer is suitable for most electroporation experiments, but we recommend using Neon NxT Resuspension T Buffer for electroporation at or above 1,900 V. Neon NxT Resuspension Genome Editing Buffer is designed for genome editing experiments, particularly for exceptional knock-in efficiency. Neon NxT Resuspension T Buffer is the only buffer in the kit that contains animal-origin components.

Q: Why should I use Neon NxT buffer tubes no more than 12 times?
A: The biggest concern is cross-contamination. We strongly recommend using a new tube for each type of payload or cell.

Q: Do I have to use 8 tips to use the 8-channel pipette?
A: No, you can use as few tips as you would like on the 8-channel pipette, including using 4 and alternating them to use with a 24-well plate. However, it is recommended that for using less than 4 tips, the 1-channel pipette be used. This will help provide a better electroporation experience, conserve 8-channel buffer tubes, and prevent cross-contamination.

Q: Do I need to turn off the device to switch the pipette stations?
A: No, you can simply unplug the gray connection cord from one station and plug another station into the device while leaving it running.

Specifications

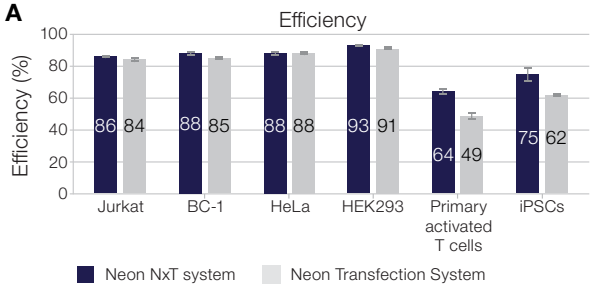
Comparison of the Neon NxT Electroporation System and the Neon Transfection System.

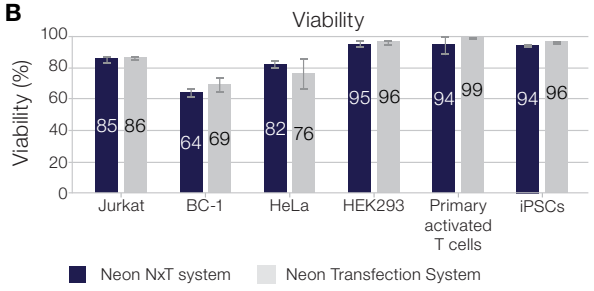
Specification	Neon NxT Electroporation System	Neon Transfection System
Pipette type	1-channel and 8-channel	1-channel
Electroporation volume	10 µL or 100 µL	
Electroporation buffer volume*	2 mL	3 mL
Tip attachment	ClipTip technology	Friction
Electroporation pulses	1–10	
Pulse duration	1–100 ms	
Pulse voltage	500–2,500 V	
Arc detection	Yes	No
Cloud connectivity	Yes	No
Device dimensions**	9.5 x 7.6 x 10.1 in. (W x H x D) 11.9 lb (5.4 kg)	9.5 x 8.9 x 13.6 in. (W x H x D) 13.8 lb (6.25 kg)
Cable management feature†	Yes	No
Touch display	8-inch capacitive touchscreen	7-inch touchscreen
Electrical rating	100–240 VAC, 270 W	100–240 VAC, 150 W

* The buffer tube of the Neon NxT Electroporation System has a 2 mL level indicator.
** The Neon NxT pulse generator can be moved into or out of a typical BSC without removing the sash.
† Excess cable length can be secured behind the Neon NxT system with the attachable cable organizer.

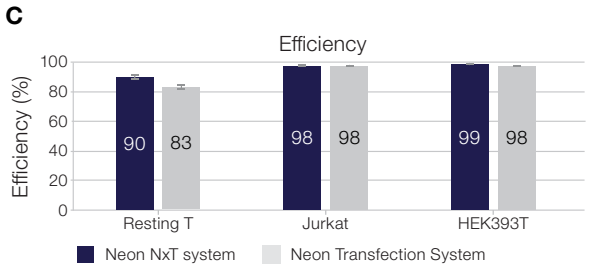
Same technology, same performance

GFP DNA 10 µL





GFP mRNA 100 µL



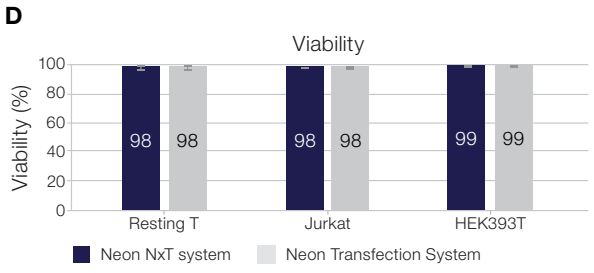


Figure 7. Performance of the Neon NxT Electroporation System and the Neon Transfection System. Performance was evaluated by transfecting different mammalian cell lines with GFP plasmid DNA or GFP mRNA. **(A)** GFP plasmid DNA transfection efficiency reported as the percentage of GFP-positive cells. **(B)** Viability of cells after transfection with GFP plasmid DNA. **(C)** GFP mRNA transfection efficiency reported as the percentage of GFP-positive cells. **(D)** Viability of cells after transfection with GFP mRNA.

Ordering information

Description	Quantity	Cat. No.
Electroporation systems		
Neon NxT Electroporation System with 1-Channel Pipette	1 system	NEON1S
Neon NxT Electroporation System Starter Pack with 1-Channel Pipette	1 system and 2 kits*	NEON1SK
Neon NxT Electroporation System with 1-Channel and 8-Channel Pipettes	1 system	NEON18S
Neon NxT Electroporation System Starter Pack with 1-Channel and 8-Channel Pipettes	1 system and 2 kits**	NEON18SK
Neon NxT Electroporation System 8-Channel Upgrade Package	1 package	NEON8/NEON8U†
Consumables		
Neon NxT Electroporation System 10 µL Kit with 1-Channel Tubes	25 x 2 reactions	N1025
Neon NxT Electroporation System 100 µL Kit with 1-Channel Tubes	25 x 2 reactions	N10025
Neon NxT Electroporation System 10 µL Kit with 1-Channel Tubes	96 x 2 reactions	N1096
Neon NxT Electroporation System 100 µL Kit with 1-Channel Tubes	96 x 2 reactions	N10096
Neon NxT Electroporation System 10 µL Kit with 8-Channel Tubes	96 x 2 reactions	N1096-8
Neon NxT Electroporation System 100 µL Kit with 8-Channel Tubes	96 x 2 reactions	N10096-8
Neon NxT Electroporation System 10 µL Kit with 8-Channel Tubes	384 x 2 reactions	N10384-8
Neon NxT Electroporation System 100 µL Kit with 8-Channel Tubes	384 x 2 reactions	N100384-8
Neon NxT 1-Channel Tubes	24 tubes	NT24
Neon NxT 1-Channel Tubes	96 tubes	NT96
Neon NxT 8-Channel Tubes	8 tubes	NT8
Accessories		
Neon NxT Electroporation System 1-Channel Pipette	1 pipette	NEON1P
Neon NxT Electroporation System 1-Channel Pipette Station	1 pipette station	NEON1PS
Neon NxT Electroporation System 8-Channel Pipette	1 pipette	NEON8P
Neon NxT Electroporation System 8-Channel Pipette Station	1 pipette station	NEON8PS

* Kits: Cat. No. N1096 and Cat. No. N10096.

** Kits: Cat. No. N1096-8 and Cat. No. N10096.

† Cat. No. NEON8 is only available in NA and EMEA. Cat. No. NEON8U is for the rest of the world.

Reference

1. Kim J-A, Cho K, Shin M-S et al. (2008) A novel electroporation method using a capillary and wire-type electrode. *Biosens Bioelectron* 23:1353–1360.

Learn more at **thermofisher.com/neonnxt**