



Electroporation

# Transfect with the best of what's NxT

## Neon NxT Electroporation System

# 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 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 saves time, helps minimize sample loss, reduces the amount of shear force experienced by cells, and mitigates the risk of sample contamination. With the surprisingly simple Neon NxT electroporation workflow, you can 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 three 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 press Electroporate. Your transfected cells are now ready for transfer to a culture vessel.

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



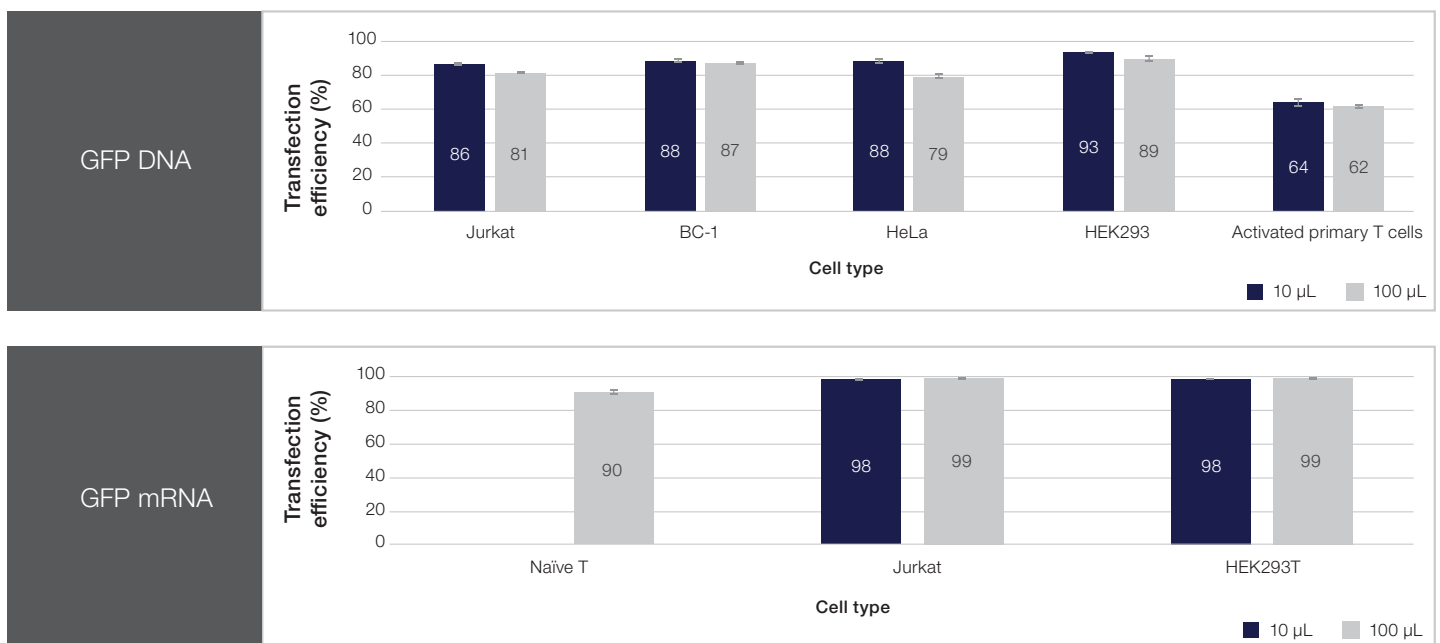
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## Proven transfection efficiency

With our proprietary electroporation tip technology, you can efficiently transfect challenging mammalian cells while maintaining high cell viability. We have observed exceptional transfection efficiency (Figure 1) and cell viability (Figure 2), even with extremely difficult cells like immune, primary, and stem cells.

### Transfection efficiency



**Figure 1. Transfection efficiency with the Neon NxT Electroporation System.** Cells were transfected with GFP plasmid DNA or GFP mRNA in 10 µL or 100 µL electroporation reactions. Transfection efficiency is reported as the percentage of GFP-positive cells (n = 3). **Note:** Naïve T cells were only electroporated in 100 µL reactions.

## Did you know?



Our electroporation tip technology has been cited in **more than 12,000 publications.**

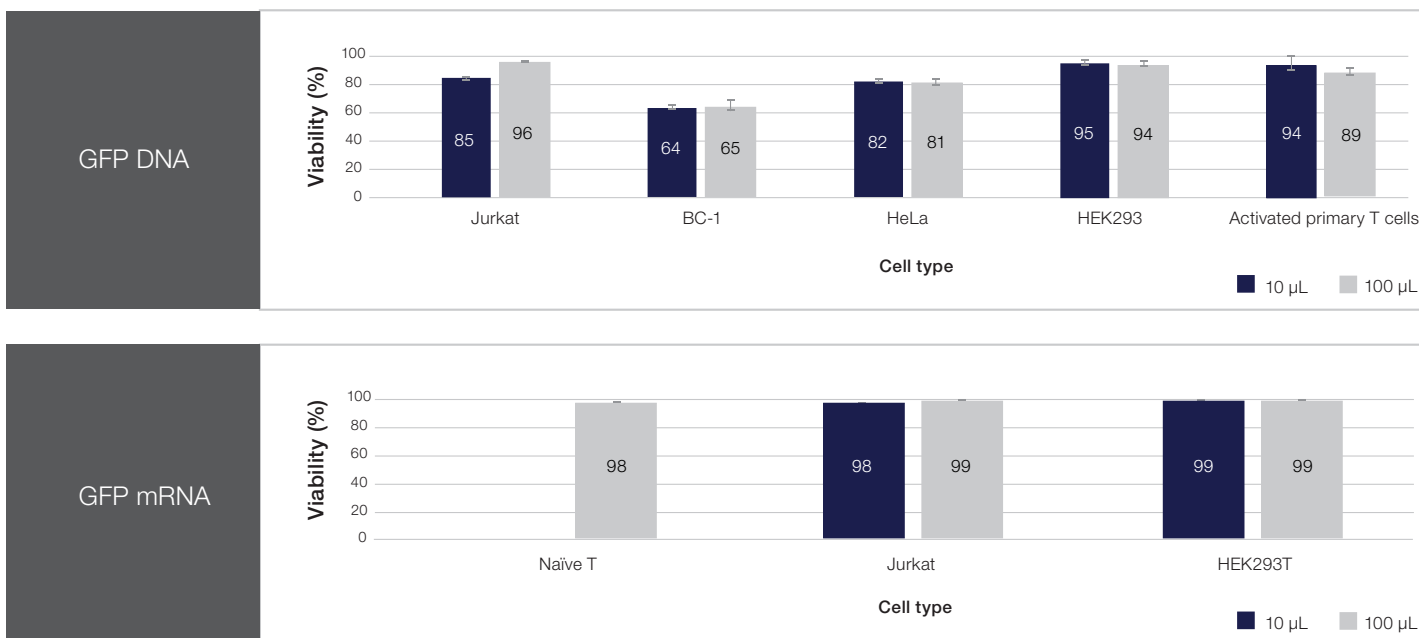


## Exceptional post-transfection cell viability

We understand how important your cells are. The Neon NxT Electroporation System enables electroporation under more controlled conditions and eliminates two pipetting steps. 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 2).

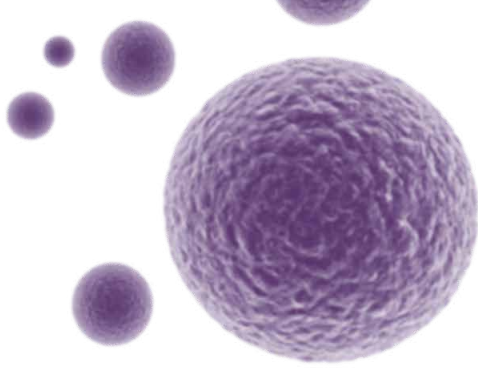
The electrodes in the Neon NxT Electroporation System are spaced widely apart and have a minimal surface area. The disposable Neon NxT pipette tip houses one of the electrodes, while the other is located at the bottom of the Neon NxT buffer tube. The small surface area creates a more uniform electric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

### Viability



**Figure 2. Cell viability following electroporation on the Neon NxT system.** Cells were transfected with GFP plasmid DNA or GFP mRNA in 10 µL or 100 µL electroporation reactions. 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. **Note:** Naive T cells were only electroporated in 100 µL reactions.





**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 will enable you to identify unsuccessfully processed samples.



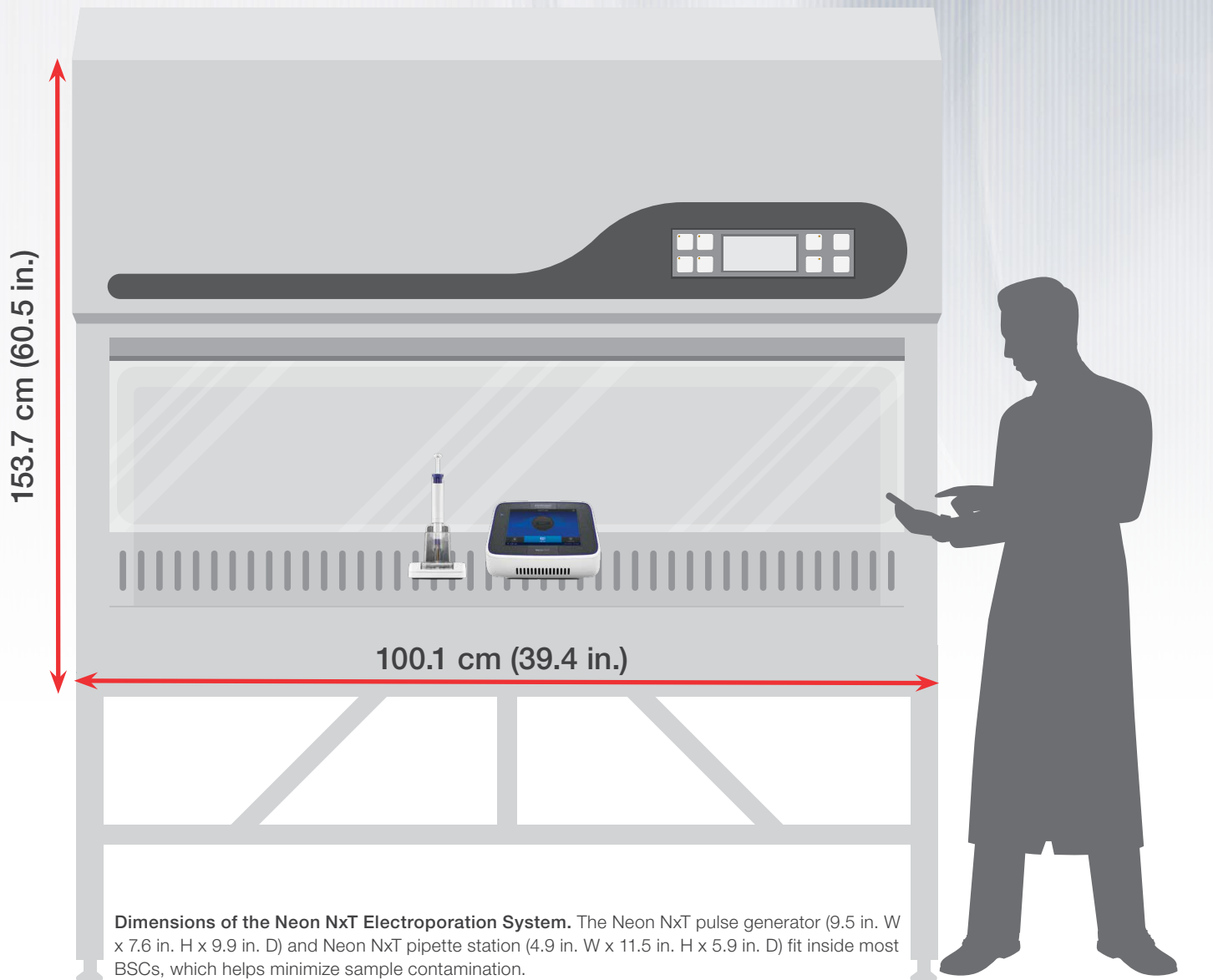
# Preserve your precious samples

## 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 to 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 ensures your samples stay within the aseptic area at all times while using the instrument, reducing the risk of contaminating precious cells. The Neon NxT system also has a new cable management feature that makes using it in the BSC hassle-free.



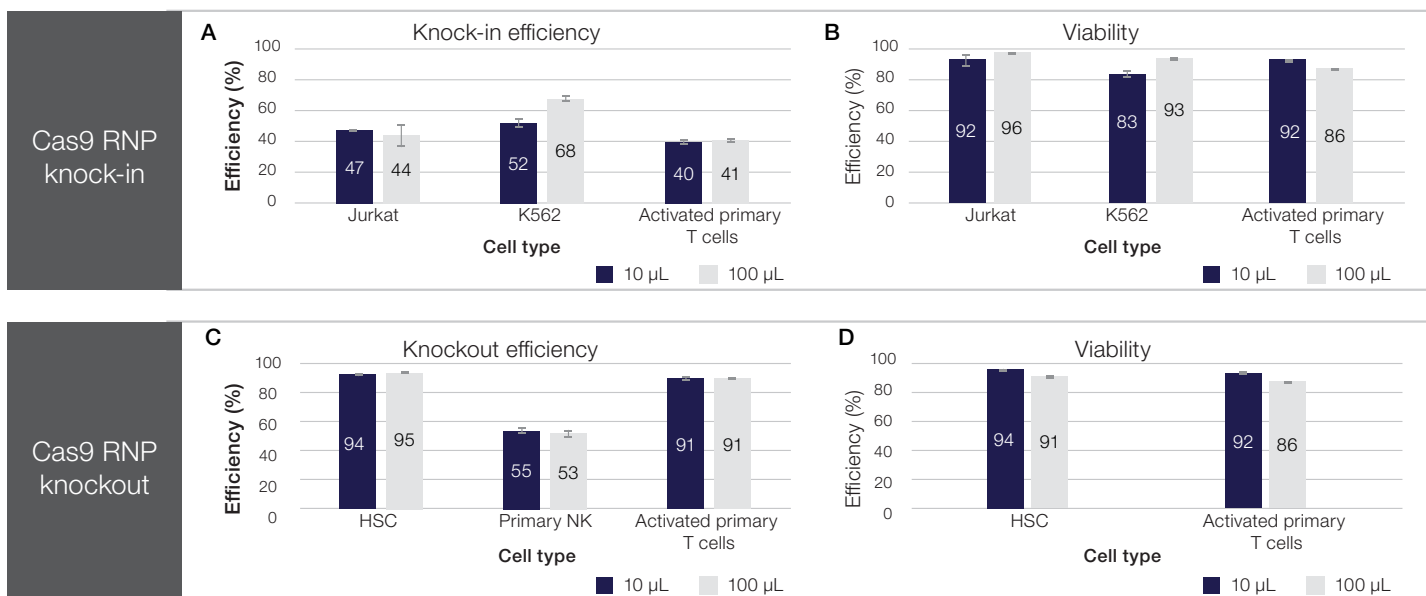
## Enhance genome editing efficiency

Genome editing holds great promise in the pursuit of a greater understanding of human health and disease, including the transformation of 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 3). 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](https://thermofisher.com/geneediting).



**Figure 3. Performance of Neon NxT Resuspension Genome Editing Buffer in CRISPR-Cas9–based genome editing experiments 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  $\mu$ L or 100  $\mu$ L 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. For primary NK cells, indel efficiency (%), determined through a genomic cleavage detection (GCD) assay, served as a proxy for knockout efficiency. **(D)** Post-electroporation viability of knockout cells.

## 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.





# 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 improves 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?

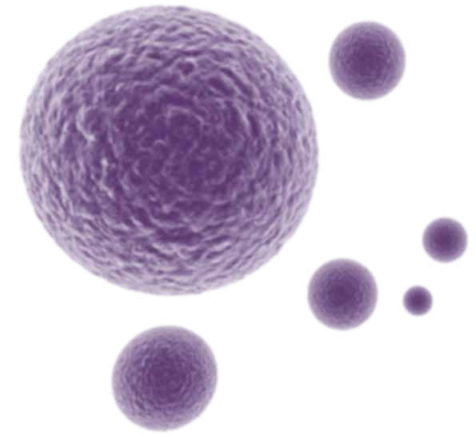


With **Thermo Scientific™ ClipTip™ technology**, the Neon NxT pipette tip will click when it is in place. The low forces involved in tip attachment and ejection also provide an **ergonomic benefit**.



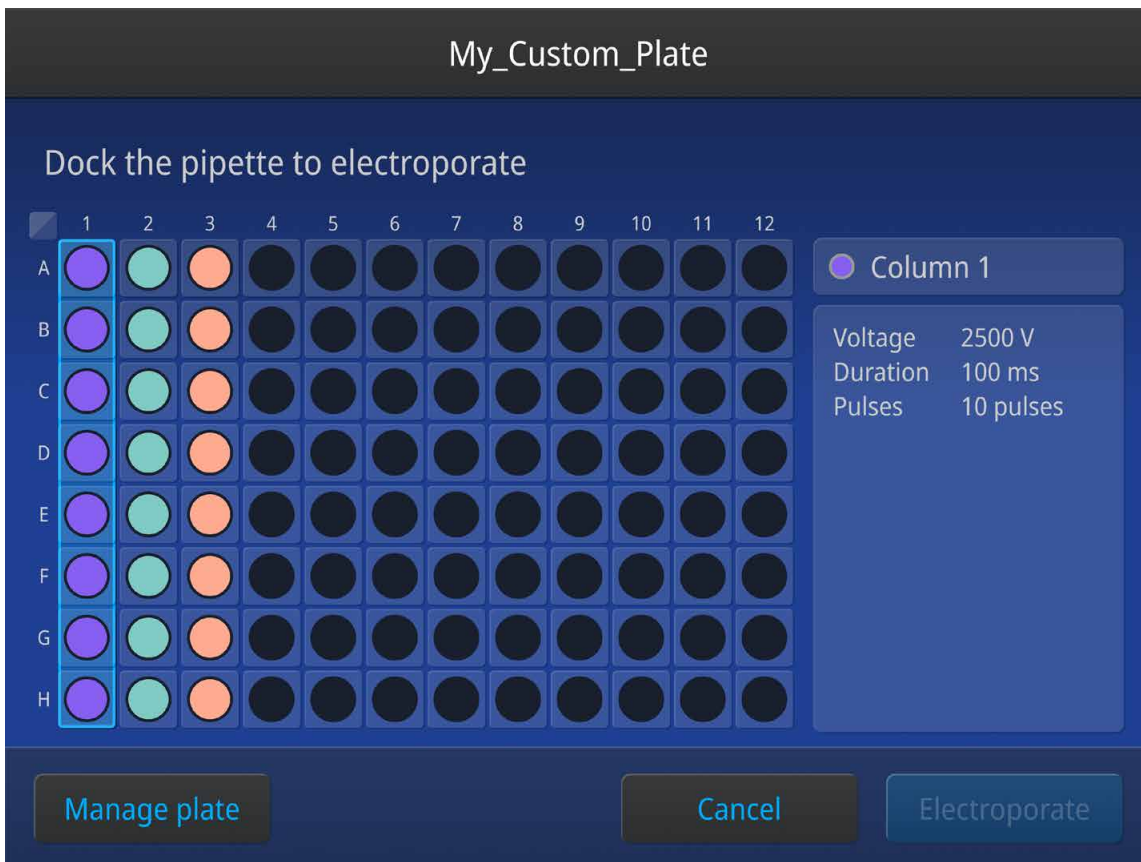
## One buffer kit for many mammalian cell types

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. We recommend using Invitrogen™ Neon™ NxT Resuspension R Buffer at voltages below 1,900 V and Invitrogen™ Neon™ NxT Resuspension T Buffer at higher voltages. Also included in the kit is Neon NxT Resuspension Genome Editing Buffer that allows for exceptional genome editing efficiency. Transfect popular cell lines with our optimized protocols, or follow our standard optimization procedure for new cell lines.



## Time-saving plate setup

Plate setup mode takes efficiency to another level. 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?



With the Neon NxT system, you can electroporate **up to 24 samples** in less than **20 minutes**.



## 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
- Cell type
- Pulse width
- Buffer type
- Number of pulses
- 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  $1 \times 10^4$  to  $1 \times 10^7$  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.

### Plan with ease using the TransfectionLab cloud-based app

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

## Did you know?



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

## Did you know?

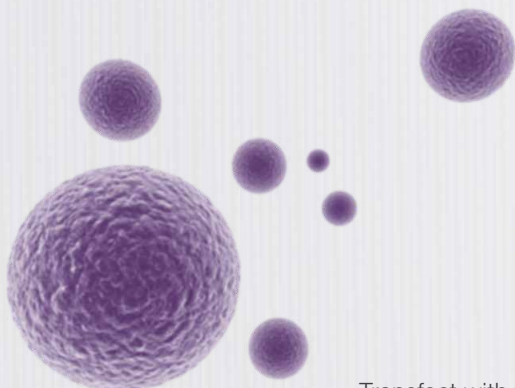


The Invitrogen™ **TransfectionSelect™** tool filters the **protocol library** based on the criteria you enter, so you can find the most appropriate protocol for your application.

## Did you know?

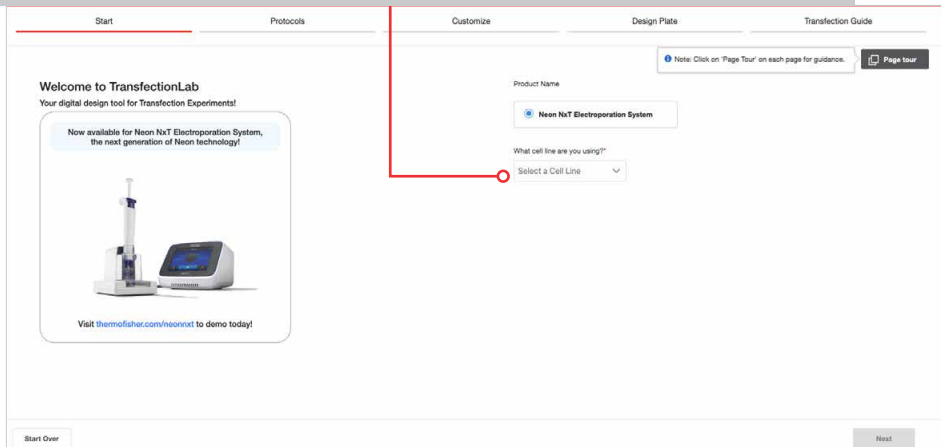


You can **remotely set up** multiple plate layouts for up to 384 samples, and any protocol or plate layout saved in the app can be accessed through the cloud rather than the user interface of the Neon NxT system.

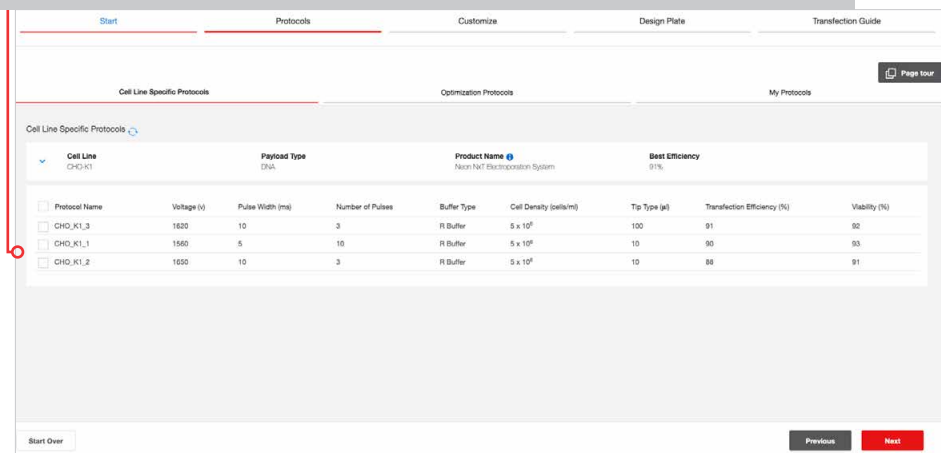


# Experimental design with the TransfectionLab app

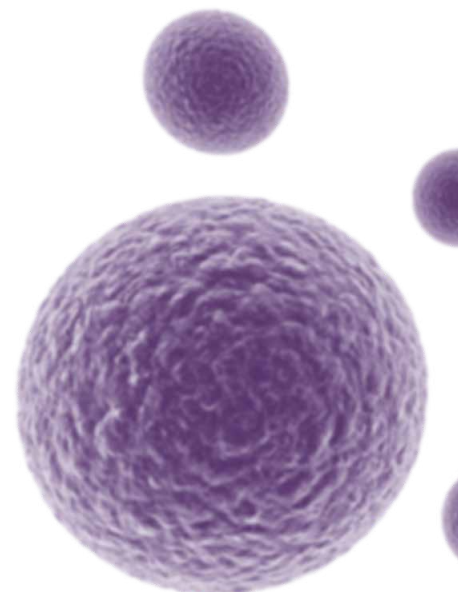
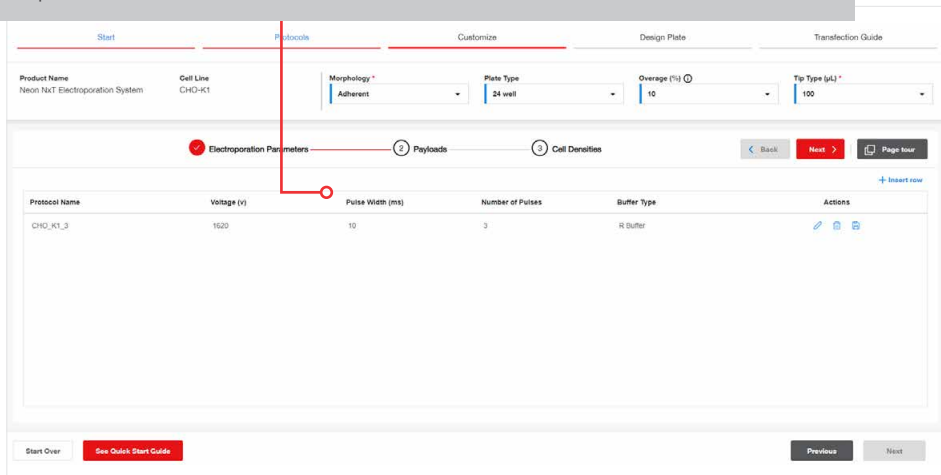
**Start tab:** Enter your cell type. The app will filter the protocol library based on your input.



**Protocols tab:** Select your protocol. You can choose a cell line-specific protocol or create your own.



**Customize tab:** Enter the cell density and cell type, and the working and stock concentration of your payload. You can choose single-payload delivery or adjust settings for CRISPR-Cas9 applications to perform up to three knock-in or knockout experiments.



**Design Plate tab:** Design multiple plates for up to 384 samples through the visually appealing and interactive interface.

The Design Plate interface features a navigation bar with tabs: Start, Protocols, Customize, Design Plate, and Transfection Guide. The main area displays a 24-well plate layout (4 rows x 6 columns) for 'Plate\_1'. Wells are numbered 1-6 across rows A-D. A red callout box states: 'On the Design Plate page, you will be able to complete your experiment design by laying out your plates. Add additional plates for your experiment using the "Add" button below (up to 384 wells). Next Skip Tour'. Below the plate are 'Add', 'Rename', and 'Save' buttons. To the right, a table shows the configuration for each payload:

Legend	Payload Name	Working Concentration (Weight/Volume)	Action
1	No Payload Control	0	<a href="#">Apply</a>
2	DNA1	1	<a href="#">Apply</a>
3	DNA2	2	<a href="#">Apply</a>

Additional buttons include 'Clear from well(s)', 'Start Over', 'See Quick Start Guide', 'Previous', 'Save As', and 'Next'.

**Transfection Guide tab:** A personalized step-by-step guide is generated, with calculations done for you in the background, to walk you through your experiment.

The Transfection Guide interface shows a 'Materials Needed' list on the left: 1. CHO-K1 cells, 2. Payloads stored in DMEM, 3. Neon Nxt™ Transfection System, 4. Neon Nxt™ Transfection System kit, tips and tubes, 5. 24-well plate, 6. Cell counter, 7. Appropriate liquid transfer pipettes and tips. The main content is the 'Electroporation Transfection Guide' which includes a legend for 'Plate\_1' (showing the 24-well layout with payload 1 in yellow, 2 in green, and 3 in red), 'Legend Details' (Protocols: No Electroporation Control - CHO-K1, 2; Payloads: No Payload Control - 1, 2, 3; Cell Density: 1e6), and 'Culture cells: 1-2 days prior to electroporation'. It provides 'Start date and time of step' fields and a list of steps: 1. Load cells, 2. Prepare 24-well plates, 3. Add payload, 4. Harvest cells. A 'Print' button is visible. On the right, a 'Transfection Guide' sidebar shows 'Destination: Microsoft Print to PDF', 'Pages: All', 'Color: Color', and 'More settings'. At the bottom are 'Back to home page', 'Previous', and 'Download' buttons.



## 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 new features that make it easier to use, as well as a new buffer designed for genome editing applications. 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 4.

**Q: What are the advantages of the Neon NxT electrode design?**

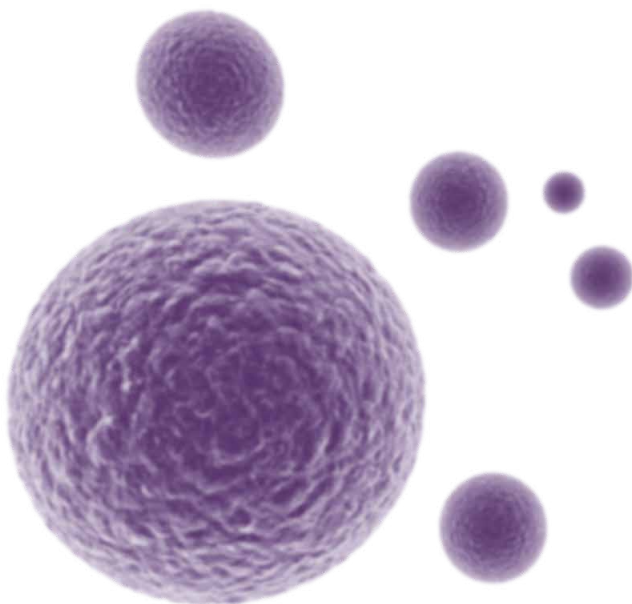
**A:** The gold-coated electrodes produce a more uniform electrical field and a lower pH gradient across cell suspensions. The unique design helps maintain physiological conditions, so cells have much higher survival rates after electroporation than cells subjected to conventional electroporation [1].

**Q: How do you calibrate the Neon NxT pipette?**

**A:** The Neon NxT pipette is permanently calibrated by the manufacturer, so you do not need to calibrate it 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 Neon NxT kits work with the Neon system and vice versa?**

**A:** Neon NxT E10, E100, R, and T buffers have the same compositions as Neon buffers E, E2, R, and T, respectively. However, Neon NxT tips and tubes have a different design that improves usability, and therefore are not compatible with the Neon Transfection System.



# Specifications



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

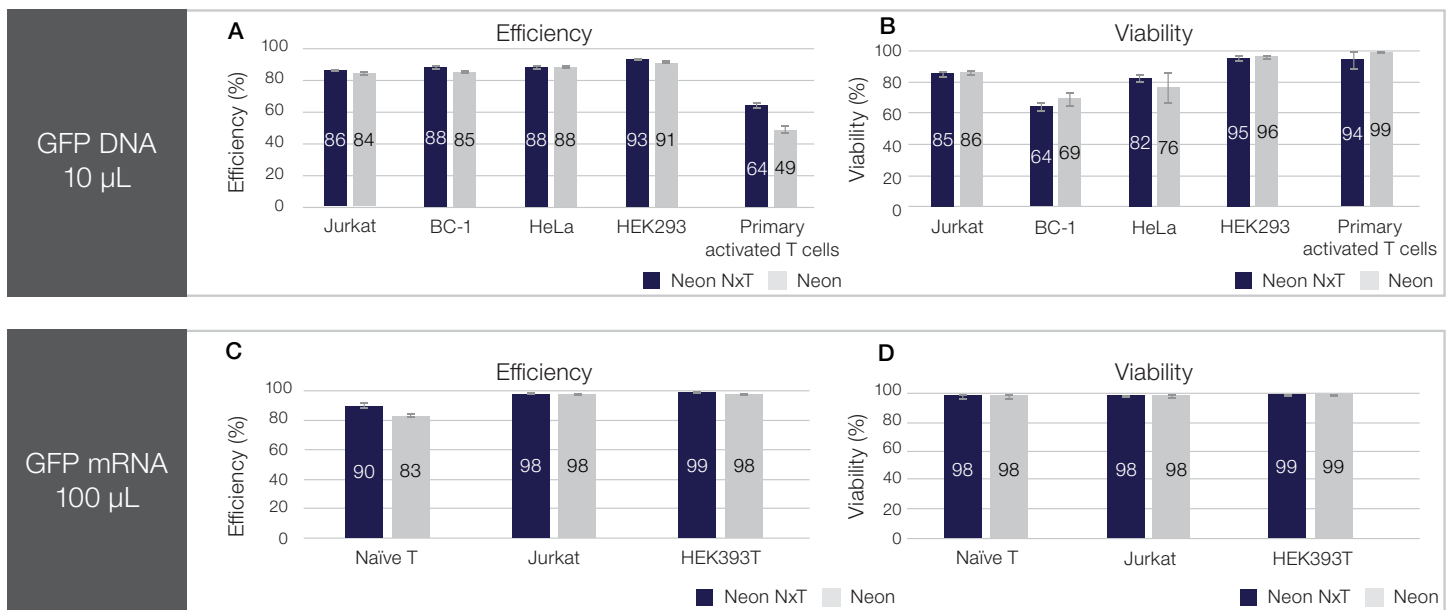
Specification	Neon NxT Electroporation System	Neon Transfection System
Electroporation volume	10 $\mu$ L or 100 $\mu$ L	10 $\mu$ L or 100 $\mu$ L
Electroporation buffer volume*	2 mL	3 mL
Tip attachment	ClipTip technology	Friction
Electroporation pulses	1–10	1–10
Pulse duration	1–100 ms	1–100 ms
Pulse voltage	500–2,500 V	500–2,500 V
Arc detection	Yes	No
Cloud connectivity	Yes	No
Pulse generator dimensions**	9.5 x 7.6 x 9.9 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



**Figure 4. 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.
<b>Electroporation systems</b>		
Neon NxT Electroporation System	1 system	NEON1
Neon NxT Electroporation System Starter Pack*	1 system and 2 kits**	NEON1SK
<b>Consumables</b>		
Neon NxT 10 $\mu$ L Kit	25 x 2 reactions	N1025
Neon NxT 100 $\mu$ L Kit	25 x 2 reactions	N10025
Neon NxT 10 $\mu$ L Kit	96 x 2 reactions	N1096
Neon NxT 100 $\mu$ L Kit	96 x 2 reactions	N10096
Neon NxT Tubes	96 tubes	NT96
<b>Accessories</b>		
Neon NxT Pipette	1 pipette	NEON1P
Neon NxT Pipette Station	1 pipette station	NEON1PS

\* Includes one Invitrogen™ Neon™ NxT Pipette (Cat. No. NEON1P) and the Invitrogen™ Neon™ NxT Pipette Station (Cat. No. NEON1PS).

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

## Did you know?



We have **FSE-executed IQOQ available** as an option for the Neon NxT system, to save you time and effort.







Neon NxT kit components	Neon NxT Kit (10 $\mu$ L)		Neon NxT Kit (100 $\mu$ L)	
	Cat. No. N1025 (50 reactions)	Cat. No. N1096 (192 reactions)	Cat. No. N10025 (50 reactions)	Cat. No. N10096 (192 reactions)
Tips/Tubes Kit	N1025K	N1096K	N10025K	N10096K
Neon NxT Tips	25 tips (10 $\mu$ L)	96 tips (10 $\mu$ L)	25 tips (100 $\mu$ L)	96 tips (100 $\mu$ L)
Neon NxT Tubes	8	32	8	32
Buffer Kit	N1025B	N1096B	N10025B	N10096B
Neon NxT Electrolytic E10 Buffer	50 mL	2 x 100 mL	–	–
Neon NxT Electrolytic E100 Buffer	–	–	50 mL	2 x 100 mL
Neon NxT Resuspension R Buffer	1 mL	4 x 1 mL	10 mL	4 x 10 mL
Neon NxT Resuspension T Buffer	1 mL	4 x 1 mL	10 mL	4 x 10 mL
Neon NxT Resuspension Genome Editing Buffer	1 mL	4 x 1 mL	10 mL	4 x 10 mL

#### 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](https://thermofisher.com/neonnxt)

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