Electroporation

Streamlining the electroporation workflow with the Neon NxT Electroporation System: a comparative study with the Neon Transfection System

Introduction

Cell transfection plays a vital role in altering the genetic composition or phenotypic traits of cells in various life science applications. Scientists have a diverse array of transfection methods at their disposal, allowing them to select the most appropriate approach based on the specific requirements of the application. Electroporation is a physical transfection method that involves applying an electric field to cells to create temporary pores in their membranes, allowing the introduction of foreign DNA or other molecules into the cells. It is widely used for efficient delivery of genetic material and has applications in various fields, including transgenic research, gene therapy, and cell line engineering.

The Invitrogen™ Neon™ Transfection System is a popular electroporation instrument widely used in research settings. It is known for its reliability and has a proprietary pipette tip chamber that generates a uniform electric field, leading to a significant increase in transfection efficiency and cell viability [1].

The Invitrogen™ Neon™ NxT Electroporation System is an improved version of the Neon Transfection System, featuring an improved pipette design and enhanced ergonomics while maintaining the same excellent performance. It is an advanced platform for efficiently delivering nucleic acids, proteins, and other payloads into diverse cell types. The Neon NxT system offers exceptional flexibility and customization options for genome editing, cell line development, and other transfection applications. With its innovative design and optimized parameters, the Neon NxT system enables researchers to achieve high transfection efficiency while preserving cell viability, even for challenging primary and stem cells. It incorporates Thermo Scientific™ ClipTip™ technology, ensuring secure sample fit through a unique interlocking system that guarantees a complete seal with minimal effort for attachment and removal. Additionally, the system can be seamlessly integrated with the Thermo Fisher™ Connect Platform, using the Instrument Connect application. The cloud-based Invitrogen™ TransfectionLab™ application on the Connect Platform allows for comprehensive remote experimental design for the Neon NxT Electroporation System, offering users enhanced flexibility and convenience.

Neon NxT Electroporation System

The Neon NxT system comes with several buffers in one complete kit designed to optimize electroporation efficiency for diverse types of cells and applications. These buffers are formulated to help ensure maximum cell viability and transfection efficiency, while minimizing cell stress and damage. The Invitrogen™ Neon™ NxT Electrolytic E10 Buffer, Electrolytic E100 Buffer, Resuspension R Buffer, and Resuspension T Buffer have the same compositions as the Invitrogen™ Neon™ buffers: Invitrogen™ Neon™ Electrolytic Buffer E, Electrolytic Buffer E2, Resuspension Buffer R, and Resuspension Buffer T. Neon NxT Resuspension R Buffer is optimized for efficient and gentle electroporation of mammalian cells and is ideal for transfecting a wide range of cell types, including primary cells and stem cells. The Neon NxT Resuspension 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.

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In addition, 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. This is achieved by promoting and driving the cells' natural homology-directed repair (HDR) mechanism, resulting in significantly improved knock-in performance. The GE Buffer is exclusively tailored for use with the Neon NxT Electroporation System, specifically targeting gene editing–specific payloads, such as CRISPR-Cas9. The buffer is also suitable for knock-in and knockout applications using various human cell types. Notably, the GE Buffer has shown impressive performance improvements, with knock-in performance increasing by 20–50% over standard transfection buffer (**Application note: Achieve exceptional genome editing efficiency**).

This system is also beneficial for customers looking to scale up to the Gibco™ CTS™ Xenon Electroporation System for large-scale clinical applications. The Neon NxT and CTS Xenon systems share the same core technology, which allows optimization on the Neon NxT system to scale directly to the CTS Xenon system. The Neon NxT system integrates enhanced ergonomic features and an upgraded software interface to ensure a smooth transition and flexible workflow integration for researchers migrating their experiments and workflows to the CTS Xenon system.

Here we present a comprehensive analysis comparing the electroporation performance of the Neon NxT system and the Neon system. Our study evaluated each system's efficiency in transfecting green fluorescent protein (GFP) and achieving gene knockout using multiple cell types. Results show comparable performance between the systems in terms of transfection efficiency, cell viability, and gene knockout efficiency.

Materials and methods

A wide variety of human primary and immortalized cells were electroporated using the Neon Transfection System and the Neon NxT Electroporation System. Previously optimized electroporation conditions for these cell types were used in this study (Tables 1–3). The electroporation workflows that were used are included here:

Jurkat

Jurkat cells were cultured in Gibco™ RPMI 1640 Medium with GlutaMAX™ Supplement (Cat. No. 61870036) with 10% FBS. Cells were reseeded at a concentration of 8 x 10^5 cells/mL, 24 hours prior to electroporation. Respective payload quantities and electroporation conditions are listed in Tables 1 and 2. Electroporated cells were expanded in RPMI 1640 Medium with GlutaMAX Supplement. At 24 hours post-electroporation, cells were analyzed for viability and transfection efficiency of the GFP plasmid or EGFP mRNA using the Invitrogen™ Attune™ NxT Flow Cytometer.

BC-1

BC-1 cells were cultured in Gibco™ RPMI 1640 Medium (without GlutaMAX Supplement; Cat. No. A1049101) supplemented with 10% FBS. Cells were reseeded at a concentration of 8 x 10^5 cells/mL, 24 hours prior to electroporation. Respective payload quantities and electroporation conditions are listed in Table 1. Electroporated cells were expanded in RPMI 1640 Medium. At 24 hours post-electroporation, cells were analyzed for viability and transfection efficiency of the GFP plasmid using the Attune NxT Flow Cytometer.

HeLa

HeLa cells were cultured in Gibco™ DMEM, high glucose, GlutaMAX™ Supplement, pyruvate (Cat. No. 10569010), supplemented with 10% FBS. Cells were passaged 24 hours prior to electroporation. Respective payload quantities and electroporation conditions are listed in Table 1. Electroporated cells were expanded in complete culture medium. At 24 hours post-electroporation, cells were analyzed for viability and transfection efficiency of the GFP plasmid using the Attune NxT Flow Cytometer.

Table 1. Electroporation conditions for GFP plasmid delivery.

Cell type	Cell density	Electroporation parameters	Payload	Payload amount	Buffer
Jurkat	5 x 10^7 cells/mL	1,700 V; 20 ms; 1 pulse	5 kb GFP plasmid	100 µg/mL	R
BC-1	5 x 10^6 cells/mL	1,425 V; 10 ms; 4 pulses	5 kb GFP plasmid	100 µg/mL	R
HeLa	5 x 10^6 cells/mL	1,005 V; 35 ms; 2 pulses	5 kb GFP plasmid	100 µg/mL	R
HEK293	5 x 10^7 cells/mL	1,100 V; 20 ms; 2 pulses	5 kb GFP plasmid	100 µg/mL	R
Activated primary T cells	5 x 10^7 cells/mL	1,600 V; 10 ms; 3 pulses	5 kb GFP plasmid	100 µg/mL	R
iPSCs	5 x 10^7 cells/mL	1,300 V; 10 ms; 2 pulses	5 kb GFP plasmid	50 µg/mL	T

Table 2. Electroporation conditions for EGFP mRNA delivery.

Cell type	Cell density	Electroporation parameters	Payload	Payload amount	Buffer
Resting primary T cells	5 x 10^7 cells/mL	2,200 V; 20 ms; 1 pulse	EGFP mRNA	300 µg/mL	T
Jurkat	5 x 10^7 cells/mL	1,700 V; 20 ms; 1 pulse	EGFP mRNA	300 µg/mL	R
HEK293	5 x 10^7 cells/mL	1,600 V; 10 ms; 3 pulses	EGFP mRNA	300 µg/mL	R

Table 3. Electroporation conditions for RNP knockout application.

Cell type	Cell density	Target locus	Electroporation parameters	Payload amount		Buffer
				Cas9	gRNA	
HSCs	2 x 10^7 cells/mL	*B2M*	1,600 V; 10 ms; 3 pulses	120 µg/mL	28.2 µg/mL	R
Activated primary T cells	5 x 10^6 cells/mL	*TRAC*	1,600 V; 10 ms; 3 pulses	120 µg/mL	28.2 µg/mL	R
Primary NK cells	2 x 10^7 cells/mL	*B2M*	1,700 V; 20 ms; 1 pulse	120 µg/mL	32.0 µg/mL	T
iPSCs	2 x 10^7 cells/mL	*HPRT*	1,500 V; 20 ms; 1 pulse	150 µg/mL	30.0 µg/mL	R

HEK293

HEK293 cells were cultured in Gibco™ DMEM, high glucose, GlutaMAX™ Supplement, pyruvate (Cat. No. 10569010), supplemented with 10% FBS. Cells were passaged 24 hours prior to electroporation. Respective payload quantities and electroporation conditions are listed in Tables 1 and 2. Electroporated cells were expanded in complete culture medium. At 24 hours post-electroporation, cells were analyzed for viability and transfection efficiency of the GFP plasmid or EGFP mRNA using the Attune NxT Flow Cytometer.

Activated primary T cells

Peripheral blood mononuclear cells (PBMCs) were previously isolated from a leukopak using the Gibco™ CTS™ Rotea™ Counterflow Centrifugation System and frozen for future use. On day 0, PBMCs were thawed and activated using Gibco™ CTS™ Dynabeads™ CD3/CD28 beads (Cat. No. 40203D) at a 3:1 beads-to-cell ratio. The T cells were then expanded in Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM) (Cat. No A1048501) supplemented with Gibco™ CTS™ Immune Cell Serum Replacement (SR) (Cat. No. A2596101) and other components as instructed per the product insert. On day 3, activated T cells were debeaded and electroporated either for the knockout experiment or for GFP plasmid transfection. Respective payload quantities and electroporation conditions are listed in Tables 1 and 3. Electroporated cells were expanded in complete CTS OpTmizer SFM. On day 4, cells were analyzed for viability and GFP plasmid transfection efficiency using the Attune NxT Flow Cytometer. On day 6, cells were analyzed for viability and knockout efficiency using the Attune NxT Flow Cytometer.

Resting primary T cells

Peripheral blood mononuclear cells (PBMCs) were previously isolated from a leukopak using the CTS Rotea Counterflow Centrifugation System and frozen for future use. On day 0, PBMCs were thawed, and resting T cells were isolated by immunomagnetic negative selection. On the same day, electroporation was performed and the cells were cultured in RPMI 1640 Medium with GlutaMAX Supplement (Cat. No. 61870036) with 10% FBS, IL-7, and IL-15.* Respective payload quantities and electroporation conditions are listed in Table 2. On day 3, cells were analyzed for viability and EGFP mRNA transfection efficiency using the Attune NxT Flow Cytometer.

* The following media composition is recommended for users who prefer to not use FBS with resting primary T cells: CTS OpTmizer T Cell Expansion SFM (Cat. No. A1048501) + 2.6% CTS OpTmizer T Cell Expansion SFM supplement + 5% CTS Immune Cell Serum Replacement (SR) (Cat. No. A2596101) + 3% GlutaMAX Supplement (Cat. No. 35050061) + IL-7 and IL-15.

Primary hematopoietic stem cells (HSCs)

A mobilized leukopak (mLP) was used as a source of $CD34^+$ cells. To obtain $CD34^+$ cells from the mLP, an initial RBC elutriation method was used in the CTS Rotea Counterflow Centrifugation System to isolate PMBCs. Human $CD34^+$ cells were isolated from PBMCs by positive selection. Isolated $CD34^+$ cells were frozen for future use. $CD34^+$ cells were thawed 72 hours prior to electroporation and cultured at a seeding density of 2.5 x 10^4 cells/mL. After 72 hours, cells were electroporated and cultured for expansion. On day 6, cells were analyzed for viability and knockout efficiency using the Attune NxT Flow Cytometer. Respective payload quantities and electroporation conditions are listed in Table 3.

Induced pluripotent stem cells (iPSCs)

Human iPSCs were grown in Gibco™ StemFlex™ Medium (Cat. No. A3349401) in culture dishes coated with Gibco™ rhLaminin-521 (Cat. No. A29248). When the cells were 70–80% confluent, electroporation was performed using the conditions listed in Tables 1 and 3. After electroporation, cells were cultured in the same medium with additional Gibco™ RevitaCell™ Supplement (Cat. No. A26445-01) overnight. The next day, the medium was replaced with fresh StemFlex Medium (Cat. No. A3349401) without RevitaCell Supplement. Cells were analyzed for viability and GFP transfection efficiency 48 hours post-electroporation using the Attune NxT Flow Cytometer. Invitrogen™ SYTOX™ Red Dead Cell Stain (Cat. No. S34859) was used to measure cell viability. At 72 hours post-electroporation, cells were analyzed for knockout efficiency using the Invitrogen™ GeneArt™ Genomic Cleavage Detection Kit (Cat. No. A24372). Invitrogen™ PrestoBlue™ HS Cell Viability Reagent (Cat. No. P50200) was used to measure cell viability of gene knockout cells.

Primary natural killer (NK) cells

Peripheral blood mononuclear cells (PBMCs) were previously isolated from an apheresis product using the CTS Rotea Counterflow Centrifugation System and frozen for future use. NK cells were isolated from PBMCs by negative selection. NK cells were cultured and expanded for 7 days in Gibco™ CTS™ NK-Xpander™ Medium (Cat. No. A5019001). Respective payload quantities and electroporation conditions are listed in Table 3. On day 7, cells were electroporated and cultured for expansion. On day 10, 72 hours post-electroporation, cells were analyzed for knockout efficiency and viability using the Attune NxT Flow Cytometer.

Results

GFP plasmid transfection efficiency

The GFP plasmid transfection efficiency and cell viability were compared between the Neon NxT system and the previous Neon system. Figures 1 and 2 display the results for reaction volumes of 10 µL and 100 µL, respectively. The Neon NxT system showed comparable or slightly better transfection efficiency than the Neon system for both reaction volumes. Among the tested cell types, including Jurkat, BC-1, HeLa, HEK293, activated primary T cells, and iPSCs, the Neon NxT system consistently achieved higher or comparable efficiency values. Additionally, the Neon NxT system exhibited comparable cell viability to the Neon system.

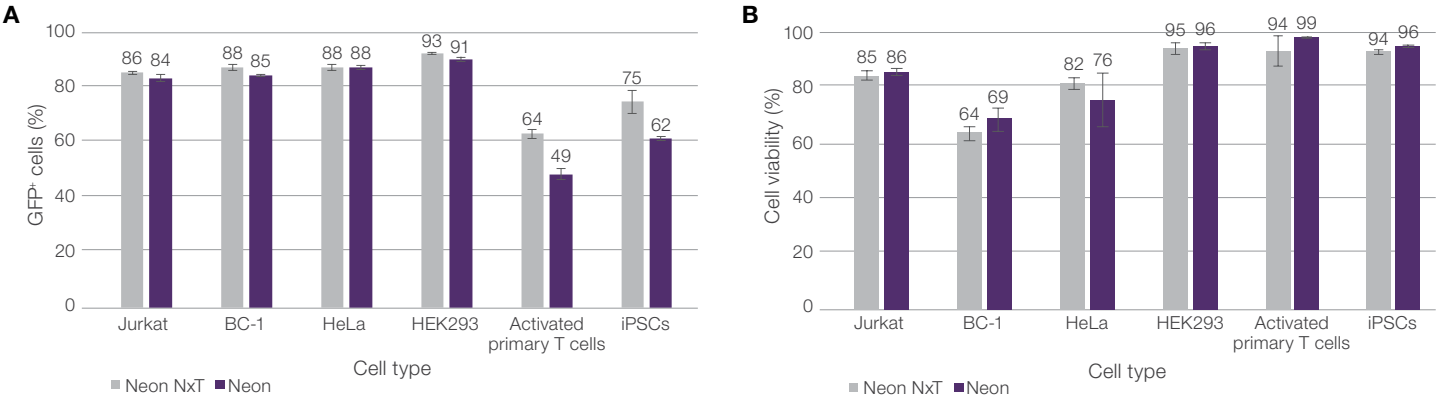

Figure 1. Comparing the Neon NxT system to the previous Neon system using 10 µL reaction volumes. (A) GFP plasmid transfection efficiency (5 kb GFP plasmid) and **(B)** cell viability post-electroporation of various cell types (n = 3).

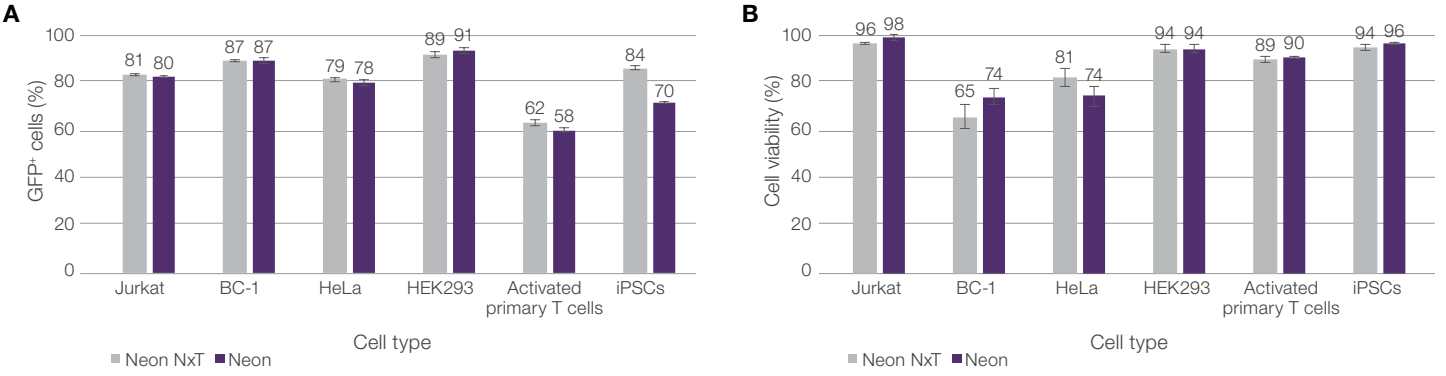

Figure 2. Comparing the Neon NxT system to the previous Neon system using 100 µL reaction volumes. (A) GFP plasmid transfection efficiency (5 kb GFP plasmid) and **(B)** cell viability post-electroporation of various cell types (n = 3).

EGFP mRNA transfection efficiency

The electroporation transfection efficiency and cell viability results for EGFP mRNA transfection using the two different instruments, the Neon NxT system and the Neon system, are presented in Figure 3 for 10 µL reaction volumes and Figure 4 for 100 µL reaction volumes.

Transfection efficiencies for resting T cells, Jurkat, and HEK293 cells were comparable for both reaction volumes between the Neon NxT and Neon systems. The transfection efficiency for resting T cells was relatively higher using the Neon NxT system (90%) as compared to the Neon system (83%). High efficiency was observed in Jurkat and HEK293 cells, with >98% EGFP mRNA transfection obtained for both reaction volumes.

Additionally, consistent viability results indicate that both instruments have equivalent performance across different cell types. High cell viability was observed for all the cell lines with both instruments at both reaction volumes, with 98–99% viability.

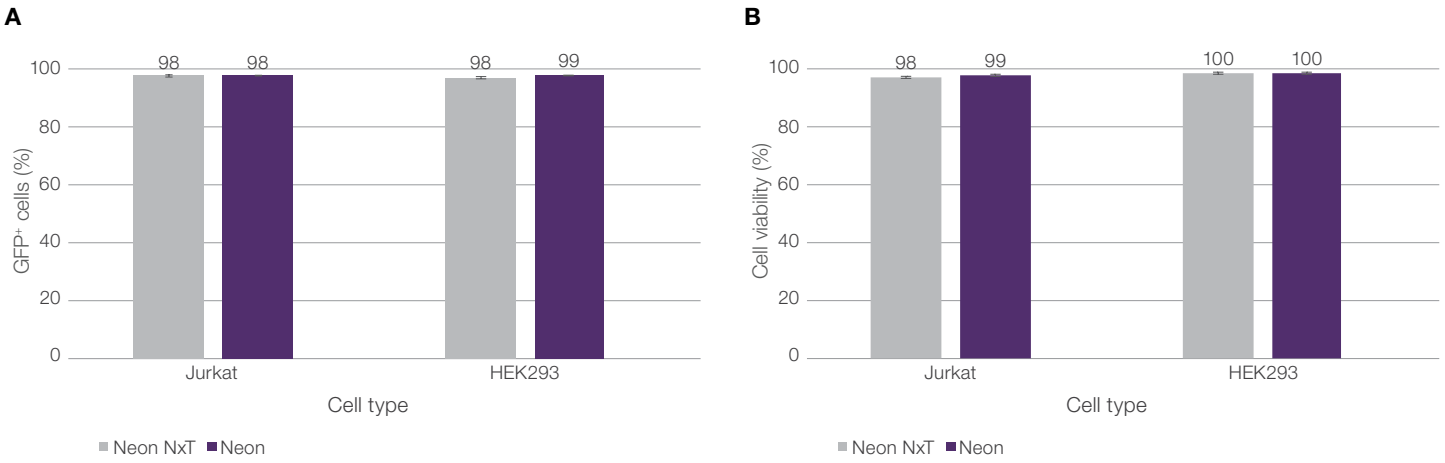

Figure 3. Comparing the Neon NxT system to the previous Neon system using 10 µL reaction volumes. (A) EGFP mRNA transfection efficiency and **(B)** cell viability post-electroporation of various cell types (n = 3).

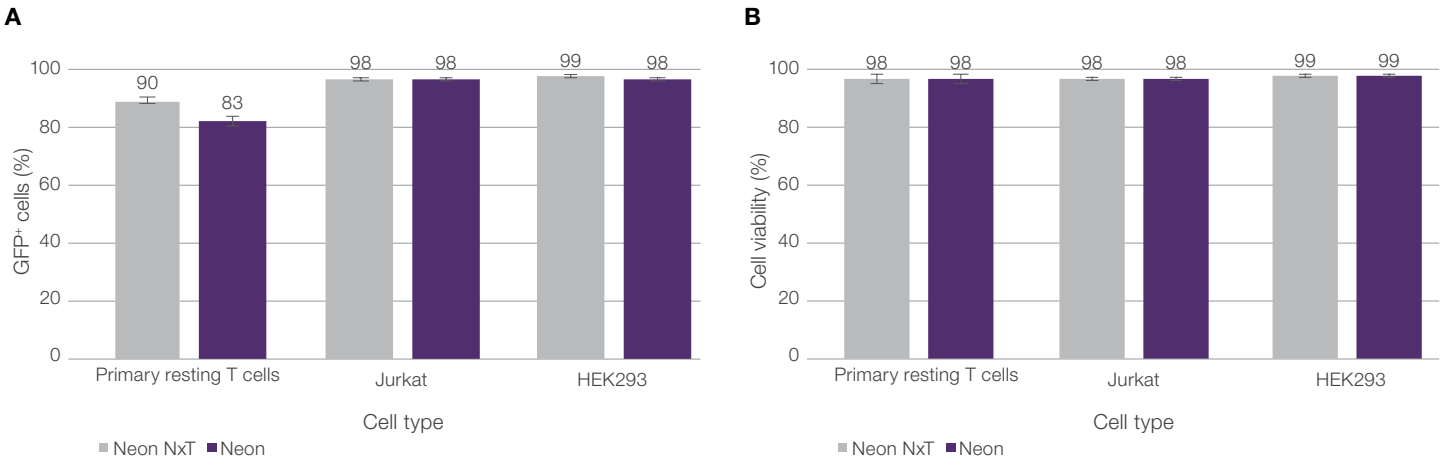

Figure 4. Comparing the Neon NxT system to the previous Neon system using 100 µL reaction volumes. (A) EGFP mRNA transfection efficiency and **(B)** cell viability post-electroporation of various cell types (n = 3).

Knockout/indel efficiency

The electroporation performance on knockout efficiency and cell viability results for various cell types using the Neon NxT system in comparison to the Neon system can be seen in Figure 5 for 10 µL reaction volumes and Figure 6 for 100 µL reaction volumes. The electroporation performance on indel efficiency and cell viability results for human iPSCs using the Neon NxT system in comparison to the Neon system can be seen in Figure 7.

For both reaction volumes, the Neon NxT system showed similar, if not slightly improved, knockout efficiency compared to the Neon system. Across the tested cell types, the average knockout efficiency for Neon NxT was comparable to or slightly higher than that of Neon system. The standard deviations remained within acceptable ranges for both systems, indicating consistent performance.

The Neon NxT system showed comparable results to the Neon system regarding cell viability. Most cell types demonstrated similar average viability on both instruments, indicating that high viability was maintained following knockout using the Neon NxT system.

Electroporation was performed with human iPSCs in 10 µL reaction volumes, and indel efficiency was measured using a GCD assay. The Neon NxT system demonstrated comparable indel efficiency and viability to the Neon system.

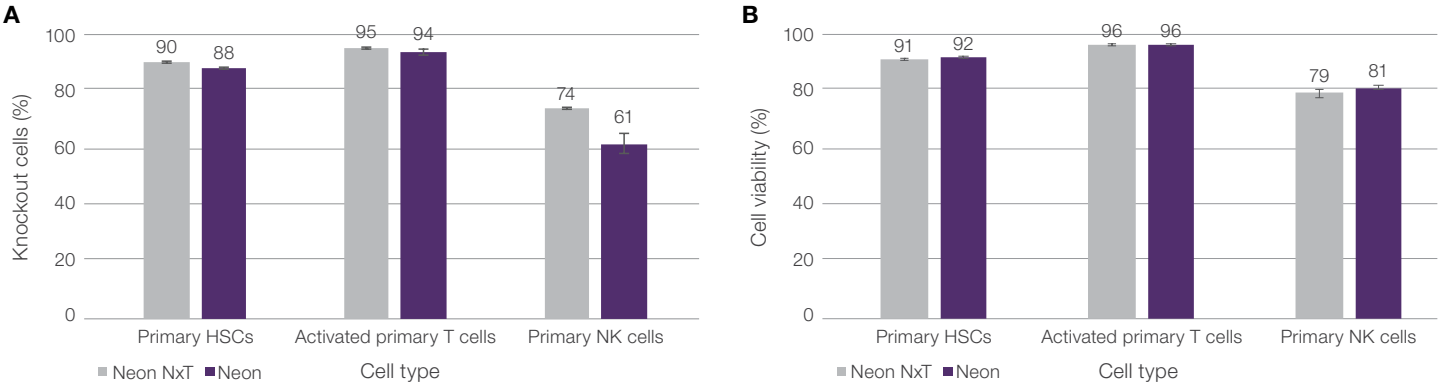

Figure 5. Comparing the Neon NxT system to the previous Neon system using 10 µL reaction volumes. (A) CRISPR-Cas9–mediated gene knockout efficiency and **(B)** cell viability post-electroporation of various cell types (n = 3).

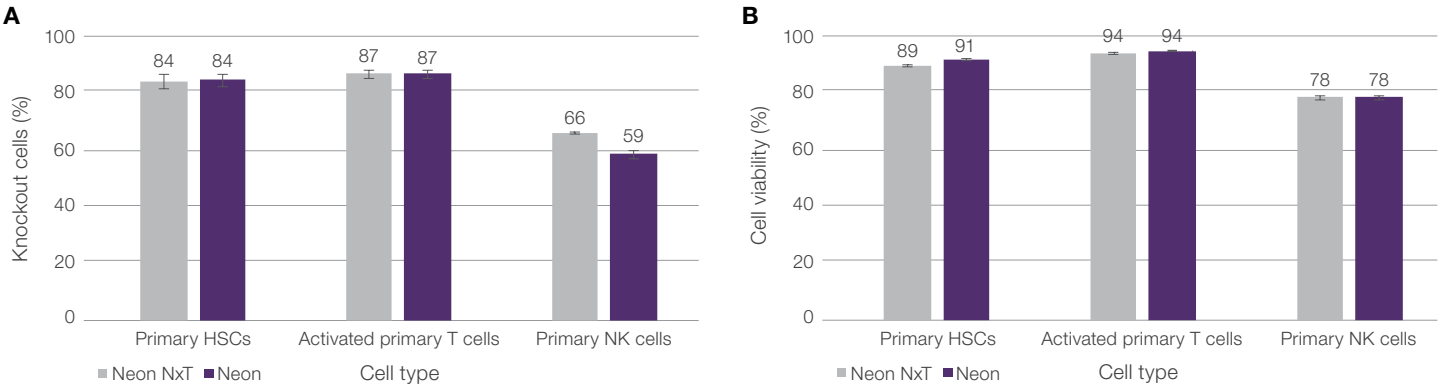

Figure 6. Comparing the Neon NxT system to the previous Neon system using 100 µL reaction volumes. (A) CRISPR-Cas9–mediated gene knockout efficiency and **(B)** cell viability post-electroporation of various cell types (n = 3).

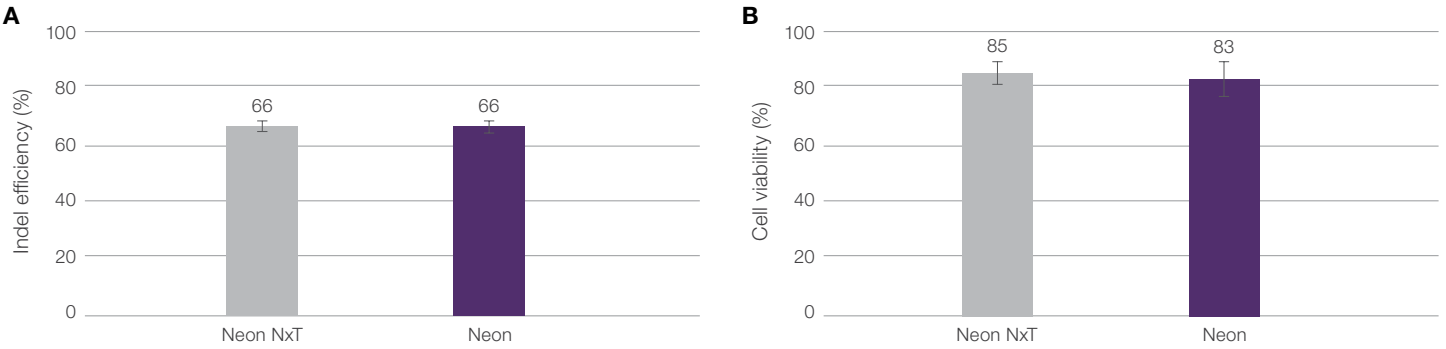

Figure 7. Comparing the Neon NxT system to the previous Neon system at 10 µL reaction volume. (A) CRISPR-Cas9–mediated gene indel efficiency and **(B)** cell viability post-electroporation of human iPSCs (n = 8).

Table 4. Comparison of the Neon NxT Electroporation System and the Neon Transfection System.

Specification	Neon NxT Electroporation System	Neon Transfection System
Electroporation volume	10 µL or 100 µL	10 µL or 100 µL
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 pulse generator on the Neon NxT Electroporation System 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.

Conclusions

In this study, we conducted a comparative analysis of the electroporation performance between the Neon NxT and the previous Neon system for nine cell types. The results indicate that both Neon NxT and Neon instruments have similar performance in terms of GFP plasmid and mRNA transfection efficiency, as well as gene knockout efficiency. The Neon NxT system is the improved next generation of the Neon system, offering researchers a reliable tool for electroporation. With its comparable or slightly enhanced performance, researchers can confidently use the Neon NxT system, expecting similar or improved electroporation efficiency and maintaining high cell viability.

The Neon NxT Electroporation System is an advanced and versatile platform that offers several benefits compared to the previous Neon system (Table 4). Its advanced features and user-friendly interface make it a valuable tool for researchers in fields ranging from basic biology to drug development. The improved Neon NxT system provides equivalent efficiency, reproducibility, flexibility, and ease of use for various applications, such as CRISPR-Cas9 genome editing, gene expression, and cell line development.

Reference

1. Kim JA, Cho K, Shin MS, et al. (2008) A novel electroporation method using a capillary and wire-type electrode. *Biosens Bioelectron* 23(9):1353–1360.

Ordering information

Product	Quantity	Cat. No.
Neon NxT Electroporation System	1 system	NEON1
Neon NxT Electroporation System Starter Pack	1 kit	NEON1SK
Neon NxT Electroporation System 10 µL Kit	25 x 2 reactions	N1025
Neon NxT Electroporation System 100 µL Kit	25 x 2 reactions	N10025
Neon NxT Electroporation System 10 µL Kit	96 x 2 reactions	N1096
Neon NxT Electroporation System 100 µL Kit	96 x 2 reactions	N10096
Neon NxT Electroporation System Tubes	96 tubes	NT96
Neon NxT Electroporation System Pipette	1 pipette	NEON1P
Neon NxT Electroporation System Pipette Station	1 station	NEON1PS
Gibco Fetal Bovine Serum, value, heat inactivated	500 mL	A5256801
RPMI 1640 Medium, GlutaMAX Supplement	500 mL	61870036
CTS NK-Xpander Medium	500 mL	A5019001
RPMI 1640 Medium (ATCC modification)	500 mL	A1049101
DMEM, high glucose, GlutaMAX Supplement, pyruvate	500 mL	10569010
CTS Dynabeads CD3/CD28	10 mL	40203D
CTS OpTmizer T Cell Expansion Serum-Free Medium (SFM)	1,000 mL	A1048501
CTS Immune Cell Serum Replacement	50 mL	A2596101
TrueCut Cas9 Protein v2	500 µg	A36499
TCR Alpha/Beta Monoclonal Antibody (IP26), eFluor 450, eBioscience	100 tests	48-9986-42
CD56 (NCAM) Monoclonal Antibody (TULY56), PE, eBioscience	100 tests	12-0566-42
Beta-2 Microglobulin Monoclonal Antibody (B2M-01), PE	100 tests	MA1-19641
GlutaMAX Supplement	100 mL	35050061
StemFlex Medium	500 mL	A3349401
rhLaminin-521	100 µg	A29248
RevitaCell Supplement	5 mL	A26445-01
SYTOX Red Dead Cell Stain	1 mL	S34859
GeneArt Genomic Cleavage Detection Kit	20 reactions	A24372
PrestoBlue HS Cell Viability Reagent	25 mL	P50200
CTS Rotea Counterflow Centrifugation System	1 system + services	A47695
Attune NxT Flow Cytometer, blue/violet/yellow	1 system	A24859

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