Electroporation

Achieve exceptional genome editing efficiency

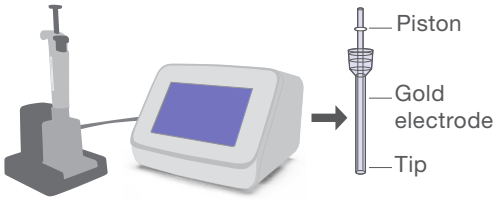
Utilizing the Neon NxT Resuspension Genome Editing Buffer with the Neon NxT Electroporation System

Introduction

Genome engineering is a powerful tool that enables researchers to study, alter, create, and reconstitute highly complex pathways, DNA sequences, genes, and natural biological systems. Editing endogenous genomic DNA (gDNA) in eukaryotic cells has been revolutionized with the advent of CRISPR (clustered regularly interspaced short palindromic repeats)-Cas9 technology. To introduce a specific edit into the DNA, such as introducing a single-nucleotide polymorphism (SNP) or a disease-specific mutation, we can exploit the cell's homology-directed repair (HDR) pathway. By introducing a short donor DNA sequence along with the CRISPR-Cas9/guide RNA (gRNA) complex, we can drive the cell to use the donor DNA to repair the double-stranded break.

Electroporation is one of the most versatile and highly efficient methods for delivering exogenous DNA into mammalian cells. This transfer of macromolecules from the extracellular space is achieved in the presence of an electric current that forms temporary pores in the cell membrane.

The new Invitrogen™ Neon™ NxT Electroporation System with the Neon™ NxT Resuspension Genome Editing (GE) Buffer can help overcome the challenges of genome editing in difficult-to-transfect cell models. The GE Buffer is formulated specifically for use with the Neon NxT Electroporation System to improve performance with gene editing–specific payloads (e.g., CRISPR-Cas9) for knock-in (KI)- or knockout (KO)-based)-based applications in a variety of human primary cells and cell lines. Experimental testing in five immune cell models for either KO or KI editing with Invitrogen™ TrueCut™ Cas9 Protein v2 showed that the Neon NxT system with GE Buffer had exceptional performance for KI and slightly improved performance for KO, compared to the original Invitrogen™ Neon™ Transfection System with Resuspension Buffer R.



Proprietary electroporation tip technology

The Neon NxT Electroporation System is the next generation of electroporation, leveraging the unique and trusted Neon™ electroporation technology that increases transfection efficiency and cell viability. The innovative design of the pipette tip maximizes the distance between the two electrodes while minimizing their surface area. This allows the sample to experience a more uniform electrical field with minimal pH change, less ion formation, and negligible heat generation.

Neon NxT Electroporation System

Driven by the unique proprietary pipette tip technology, the Neon NxT Electroporation System demonstrates exceptional transfection efficiency and high post-transfection cell viability. It also demonstrates impressive genome editing efficiency with the use of the Resuspension Genome Editing Buffer. With a streamlined 3-step workflow, digital connectivity, and a user-friendly interface, the Neon NxT Electroporation System can help put you in control of your experiments.

Features of the Neon NxT Electroporation System

- **Small footprint**—benchtop design that fits inside tissue culture hood, reducing contamination risk
- **Preserves samples**—minimize sample transfer loss with complete elimination of an electroporation cuvette and associated pipetting steps
- **Flexible**—deliver DNA, RNA, and protein to over 140 mammalian cell lines, and can accommodate 1 x 10^4 to 1 x 10^7 cells per reaction in a sample volume of 10 µL or 100 µL
- **Customizable**—optimize electroporation parameters freely, or leverage over 300 preprogrammed protocols with the ability to save up to 10,000 additional protocols
- **Simplicity**—workflow is easy to perform with only 3 steps; a single reagent kit is all that is required for most cell types and delivery payloads
- **Genome editing application focus**—Neon NxT Resuspension Genome Editing Buffer was specifically formulated to improve gene editing and optimized with our gene editing reagents
- **Connectivity**—Invitrogen™ TransfectionLab™ application paired with the Thermo Fisher™ Connect Platform allows for digital experiment design and connectivity to the Neon NxT system
- **Improved usability**—improved pipette ergonomics, enhanced feedback loop, and intuitive user interface with plate setup
- **Thermo Scientific™ ClipTip™ technology**—proprietary technology allows easy loading and unloading of pipette tips

Materials and methods

Overview

To demonstrate the improved performance of the Neon NxT Resuspension Genome Editing (GE) Buffer, the Neon NxT Electroporation System with GE Buffer and the original Neon Transfection System with Resuspension Buffer R were compared on five types of immune cells. Jurkat, K562, and activated primary T cells were tested to compare the KI editing efficiency using TrueCut Cas9 Protein v2 and GFP donor dsDNA. In addition, primary hematopoietic stem cells (HSCs) and primary natural killer (NK) cells were tested to compare the knockout (KO) performance using TrueCut Cas9 Protein v2.

The general workflow for each experiment is shown in Figure 1, and materials and methods for each cell type are described below.

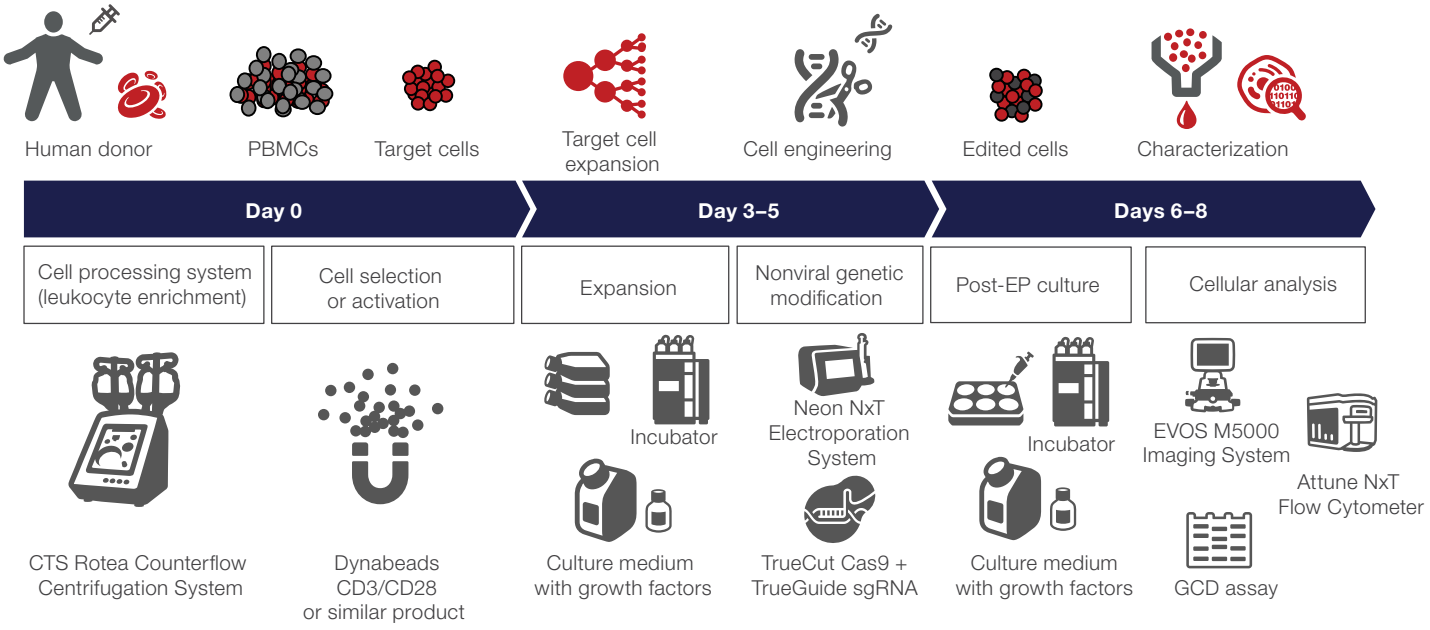


Figure 1. Genome editing workflow for primary human cells (e.g., activated T cells, HSCs, and NK cells).

Jurkat cells

Jurkat cells were cultured in Gibco™ RPMI 1640 Medium (Cat. No. 61870036) supplemented with 10% FBS. Cells were subcultured at 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 put back into culture for expansion in RPMI 1640 Medium; 72 hours post-electroporation, cells were analyzed for viability and KI efficiency, using the Invitrogen™ Attune™ NxT Flow Cytometer (Cat. No. A29004).

K562 cells

K562 cells were cultured in Gibco™ IMDM (Cat. No. 12440053) supplemented with 10% FBS. Cells were subcultured at 1.2 x 10^6 cells/mL, 24 hours prior to electroporation. Respective payload quantities and electroporation conditions are listed in Table 1. Electroporated cells were put back into culture for expansion in IMDM; 72 hours post-electroporation, cells were analyzed for viability and KI efficiency, 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 at a 3:1 ratio (beads:cells). The T cells were then expanded in Gibco™ CTS™ OpTmizer™ T Cell Expansion Serum-Free Medium (SFM) supplemented with Gibco™ CTS™ Immune Cell Serum Replacement (SR) and other components per the instructions in the product insert. After 72 hours, the activated T cells were debeaded and electroporated. The electroporated cells were put back into culture for expansion in complete CTS OpTmizer SFM. On day 6, cells were analyzed for viability and KI efficiency using the Attune NxT Flow Cytometer.

Respective payload quantities and electroporation conditions are listed in Table 1.

Table 1. Electroporation parameters for knock-in experiments.

Cell type	Cell density	Electroporation parameters	Target locus	Cas9	gRNA	GFP donor dsDNA
Jurkat	2 x 10^7 cells/mL	1,700 V; 20 ms; 1 pulse	*ACTN*	125 µg/mL	28.2 µg/mL	80 µg/mL
K562	2 x 10^7 cells/mL	1,700 V; 20 ms; 1 pulse	*ACTN*	125 µg/mL	28.2 µg/mL	80 µg/mL
Activated primary T cells	5 x 10^7 cells/mL	1,600 V; 10 ms; 3 pulses	*TRAC*	120 µg/mL	28.2 µg/mL	80 µg/mL

Hematopoietic stem cells (HSCs)

Mobilized leukopak (mLP) was used as a source of $CD34^+$ cells. To obtain $CD34^+$ cells from mLP, an initial RBC elutriation method was used in the CTS Rotea Counterflow Centrifugation System to isolate PMBCs. Human $CD34^+$ cells were isolated from the PBMCs using the Invitrogen™ Dynabeads™ CD34 Positive Isolation Kit. The isolated $CD34^+$ cells were frozen for future use. $CD34^+$ cells were thawed 72 hours prior to electroporation and seeded at a density of 2.5×10^4 cells/mL. After 72 hours, cells were electroporated and put back into culture for expansion. On day 6, cells were analyzed for viability and KO efficiency using the Attune NxT Flow Cytometer.

Respective payload quantities and electroporation conditions are listed in Table 2.

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 the PBMCs. NK cells were cultured and expanded for 5 days in Gibco™ CTS™ NK-Xpander™ Medium. On day 5, cells were electroporated and put back into culture for expansion. On day 8, 72 hours post-electroporation, cells were analyzed for indel efficiency by a genomic cleavage detection (GCD) assay.

Respective payload quantities and electroporation conditions are listed in Table 2.

Table 2. Electroporation parameters for knockout experiments.

Cell type	Cell density	Electroporation parameters	Target locus	Cas9	gRNA
HSCs	2×10^7 cells/mL	1,600 V; 10 ms; 3 pulses	*B2M*	120 µg/mL	28.2 µg/mL
Primary NK cells	2×10^7 cells/mL	1,700 V; 20 ms; 1 pulse	*AAVS1*	125 µg/mL	28.2 µg/mL

Post-electroporation analysis

The Attune NxT Flow Cytometer was used for the post-electroporation analysis of Jurkat cells, K562 cells, activated primary T cells, and HSCs. Initial cell debris was gated out in the FSC vs. SSC plot. A daughter gate was created from the initial gating to define the singlet population in the SSC-A vs. SSC-H plot.

For Jurkat and K562 cells, Invitrogen™ SYTOX™ Red Dead Cell Stain was used to distinguish viable from nonviable cells. Only viable cells staining positive for GFP were used to determine the KI efficiency.

For activated primary T cells, a PE-labeled TCR α/β antibody was used to determine the percentage of TRAC KO cells. Gating for cells negative for TCR α/β and positive for GFP was used to determine the KI efficiency.

For HSCs, a PE-labeled B2M antibody was used to determine the percentage of B2M KO cells. Gating for cells negative for SYTOX stain and positive for B2M was used to determine the percentage of KO cells, and normalized to non-electroporated control samples to determine KO efficiency.

Results and discussion

Jurkat cells, K562 cells, activated primary T cells, primary HSCs progenitor cells, and primary NK cells were genetically modified using the Neon NxT Electroporation System and the original Neon Transfection System. With the Neon NxT Electroporation System, the Resuspension Genome Editing Buffer was used, while the Resuspension Buffer R was used with the Neon Transfection System to deliver GFP donor DNA with CRISPR-Cas9/gRNA complexes. The Resuspension Genome Editing Buffer was designed to increase genome editing efficiency by driving homology-directed repair (HDR) after transfection. Jurkat, K562, and activated primary T cells were electroporated with their respective payloads to demonstrate knock-in of respective GFP donor dsDNA. Figure 2 shows the knock-in efficiency in 10 µL and 100 µL reactions. For all three cell types, the Neon NxT Electroporation System with the Resuspension Genome Editing Buffer significantly improved knock-in efficiency.

Electroporation success is a balance between transfection efficiency and cell viability. For all three cell lines (Jurkat, K562, and activated primary T cells), viability was greater than 90% (Table 3).

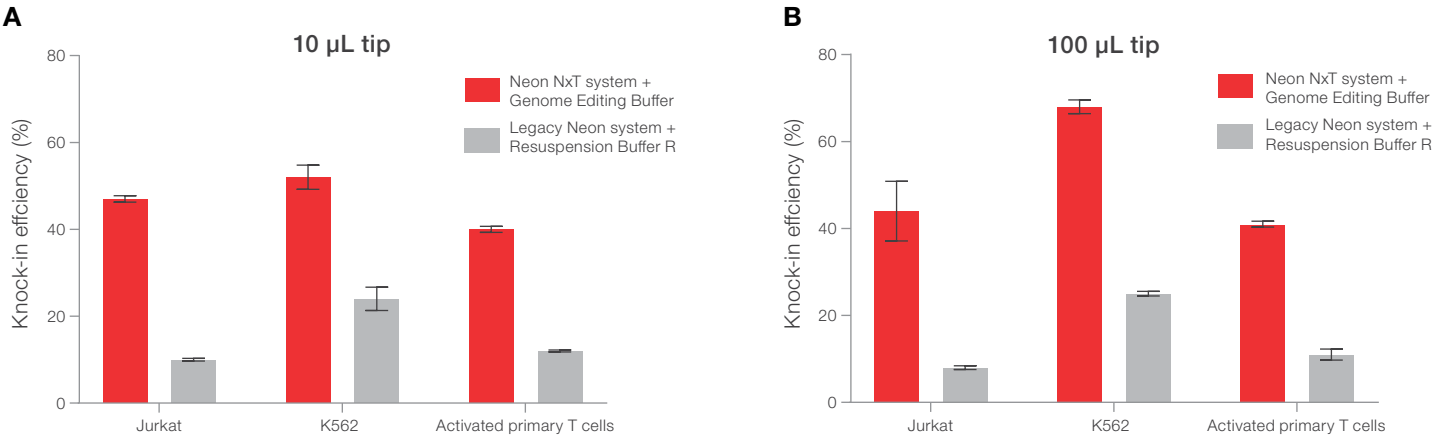


Figure 2. Comparison of knock-in efficiency. Jurkat, K562, and activated primary T cells were transfected using the Neon NxT Electroporation System with GE Buffer and the original Neon Transfection System with Buffer R at **(A)** 10 µL reaction volume, and **(B)** 100 µL reaction volume (n = 3).

Table 3. Improved knock-in efficiency utilizing the GE Buffer with the Neon NxT Electroporation System, compared to Buffer R with the original Neon Transfection System.

Cell type	Number of cells	Tip volume	Instrument and buffer	Knock-in efficiency	Cell viability
Jurkat	2×10^5	10 µL	Neon NxT system with GE Buffer	47% ± 0.73%	92% ± 3.13%
			Neon system with Buffer R	10% ± 0.30%	97% ± 0.47%
	2×10^6	100 µL	Neon NxT system with GE Buffer	44% ± 6.86%	96% ± 0.51%
			Neon system with Buffer R	8% ± 0.43%	96% ± 1.78%
K562	2×10^5	10 µL	Neon NxT system with GE Buffer	52% ± 2.78%	83% ± 1.58%
			Neon system with Buffer R	24% ± 2.68%	94% ± 1.92%
	2×10^6	100 µL	Neon NxT system with GE Buffer	68% ± 1.57%	93% ± 0.21%
			Neon system with Buffer R	25% ± 0.54%	95% ± 0.34%
Activated primary T cells	5×10^5	10 µL	Neon NxT system with GE Buffer	40% ± 0.68%	92% ± 0.91%
			Neon system with Buffer R	12% ± 0.24%	88% ± 1.08%
	5×10^6	100 µL	Neon NxT system with GE Buffer	41% ± 0.69%	86% ± 0.30%
			Neon system with Buffer R	11% ± 1.28%	83% ± 2.91%

We also used primary HSC progenitor cells and primary NK cells to compare the knockout efficiency between the Neon NxT Electroporation System with GE Buffer and the original Neon Transfection System with Buffer R. *B2M*-targeted CRISPR-Cas9 method was used to determine the knockout efficiency in $CD34^+$ (HSC progenitor) cells. The Neon NxT Electroporation System with GE Buffer improved knockout efficiency in HSC progenitor cells in both 10 µL and 100 µL reactions (Figure 3A). Even with improved genomic cleavage efficiency, viability was not affected (Table 4). Indel efficiency in primary human NK cells was also enhanced when using the Neon NxT system with GE buffer compared to the original Neon system with Buffer R (Figure 3B, Table 5).

Table 4. Improved knockout efficiency in primary human HSCs utilizing the GE Buffer with the Neon NxT Electroporation System compared to Buffer R with the original Neon Transfection System.

Cell type	Number of cells	Tip volume	Instrument and buffer	Knockout efficiency	Cell viability
HSCs	2×10^5	10 µL	Neon NxT system with GE Buffer	94% ± 0.44%	94% ± 0.31%
			Neon system with Buffer R	88% ± 0.19%	92% ± 0.24%
	2×10^6	100 µL	Neon NxT system with GE Buffer	95% ± 0.26%	91% ± 0.67%
			Neon system with Buffer R	85% ± 1.85%	91% ± 0.52%

Table 5. Improved indel efficiency in NK cells obtained using the GE Buffer with the Neon NxT Electroporation System, compared to Buffer R with the original Neon Transfection System.

Cell type	Number of cells	Tip volume	Instrument and buffer	Indel efficiency
Primary NK	2×10^5	10 µL	Neon NxT with GE Buffer	55% ± 1.77%
			Neon with Buffer R	39% ± 3.63%
	2×10^6	100 µL	Neon NxT with GE Buffer	53% ± 1.94%
			Neon with Buffer R	44% ± 5.63%

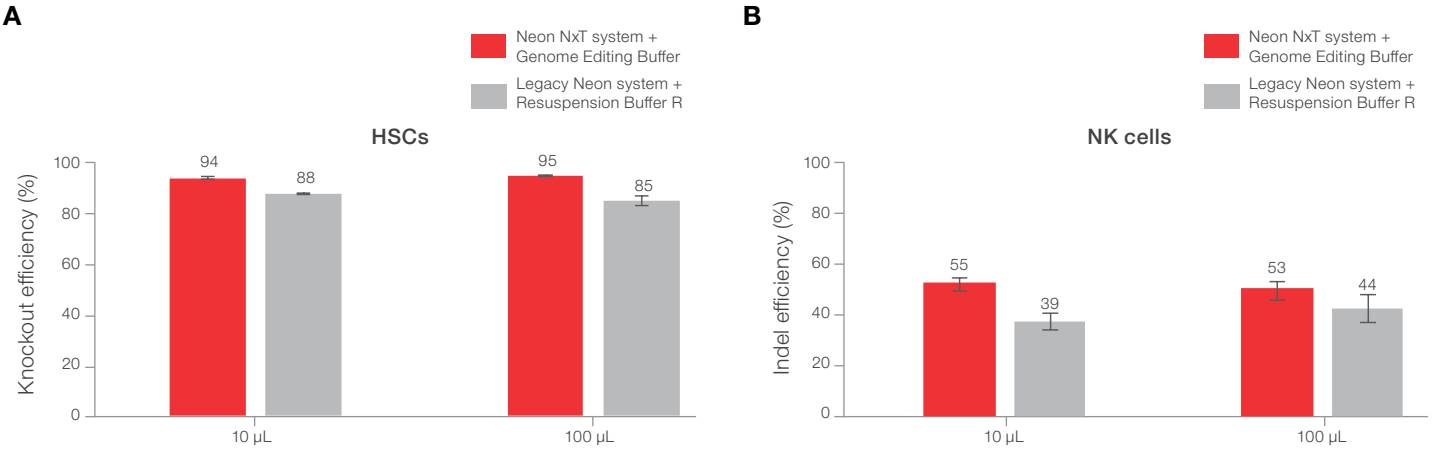


Figure 3. Comparison of knockout and indel efficiency obtained with the Neon NxT Electroporation System with GE Buffer and the original Neon Transfection System at 10 µL and 100 µL volumes. Knockout efficiency in HSCs **(A)** and indel efficiency in human primary NK cells **(B)** are reported (n = 3).

Conclusion

The Neon NxT Electroporation System with the Resuspension Genome Editing (GE) Buffer has been shown to be a powerful technology to increase KI editing efficiency in mammalian cells. As a result of improved delivery technologies and Cas9 protein designs, successful KO or gene disruption has become a more mainstream outcome. However, inserting a nonviral gene construct via HDR remains a challenge. The GE Buffer was designed and developed to improve the HDR rate in mammalian cells with the Neon NxT Electroporation System and optimized with TrueCut Cas9 protein and TrueGuide synthetic gRNA. It has been demonstrated that using the Neon NxT system with GE Buffer significantly increased KI efficiency in three difficult-to-transfect mammalian cell types. We observed a ~5-fold increase in KI efficiency in Jurkat cells using the Neon NxT System with GE Buffer as compared to the original Neon system with Buffer R. For K562 cells, KI efficiency improved approximately 2- to 2.7-fold, and for activated primary T cells, KI efficiency improved approximately 3.3- to 3.7-fold. The Neon NxT system with GE Buffer also slightly improved cleavage efficiency, as demonstrated with primary hematopoietic stem cells (HSCs) and primary natural killer (NK) cells. Overall, the Neon NxT Electroporation System with GE Buffer can provide wide utility in CRISPR-Cas9 applications by improving KI efficiency in a broad range of mammalian cells that have been traditionally difficult to genetically modify.

Ordering information

Product	Cat. No.
Neon NxT Electroporation System	NEON1
Neon NxT 10 µL Kit (includes Resuspension Genome Editing Buffer)	N1096
Neon NxT 100 µL Kit (includes Resuspension Genome Editing Buffer)	N10096
RPMI 1640 Medium, GlutaMAX Supplement	61870-036*
IDMI	12440053*
CTS NK-Xpander Medium	A5019001†
CTS OpTmizer T Cell Expansion SFM	A10221-01†
CTS Immune Cell SR	A25961-02†
FBS	16000-044
GlutaMAX Supplement (100X)	35050-061*
DPBS, no calcium, no magnesium	14190-144*
IL2 Recombinant Human Protein	CTP0023
Dynabeads Human T-Expander CD3/CD28	11141D
DynaMag-50 Magnet	12302D
TrueCut Cas9 Protein v2	A36499
CTS TrueCut Cas9 Protein	A45220†
Custom TrueGuide TRAC sgRNA	A35514
Custom TrueGuide B2M sgRNA	A35514
TrueTag Donor DNA Kit, GFP	A42992
eBioscience TCR Alpha/Beta Monoclonal Antibody (IP26), eFluor 450	48-9986-42
eBioscience CD56 (NCAM) Monoclonal Antibody (TULY56), PE	12-0566-42
GeneArt Genomic Cleavage Detection Kit	A24372
EVOS M5000 Imaging System	AMF5000
Attune NxT Flow Cytometer	A29004
CTS Rotea Counterflow Centrifugation System + 2-year service + IQOQ (installation and operational qualification)	A47695†

* For research use or further manufacturing. Not for diagnostic use or direct administration into humans or animals.

† For Research Use or Manufacturing of Cell, Gene, or Tissue-Based Products. CAUTION: Not intended for direct administration into human or animals.

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