CTS™ TrueCut™ Cas9 Protein

Catalog Numbers A45220, A45221

Pub. No. MAN0026510 Rev. B.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

Gibco™ CTS™ TrueCut™ Cas9 Protein is recombinant *Streptococcus pyogenes* Cas9 (wt) protein, purified from *E. coli*, for genome editing with CRISPR technology. Cas9 protein forms a very stable ribonucleoprotein (RNP) complex with the guide RNA (gRNA) component of the CRISPR/Cas9 system. Incorporation of nuclear localization signals (NLS) aids delivery to the nucleus, increasing the rate of genomic DNA cleavage. This protein is manufactured to meet the standards for *Ancillary Materials for Cell, Gene, and Tissue-Based Products* including USP <1043>, Ph.Eur. 5.2.12, and ISO 20399 -1, -2, -3 which include aseptic filling, and extensive release testings. It is provided at a 10 mg/mL concentration in a transfection-ready format for electroporation.

Contents and storage

Contents[1]	Cat. No.	Amount	Concentration	Storage[2]	Shelf life[3]
CTS™ TrueCut™ Cas9 Protein	A45220	2.5 mg	10 mg/mL	–20°C	24 months
	A45221	5.0 mg			

[1] Storage buffer composition: 10 mM Tris pH 8.0 (4°C), 100 mM NaCl, 200 mM Na_2SO_4, 50% glycerol

[2] Occasionally after prolonged storage at –20°C, small, clear, colorless crystals can be observed. The crystals rapidly dissipate after mild vortexing and transfer to 4°C on ice, and the crystals should be dispersed prior to formulation and transfection. No performance differences have been observed.

[3] Shelf Life duration is determined from Date of Manufacture.

Required materials not supplied

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Item	Cat. No.
CTS™ OpTmizer™ T-Cell Expansion SFM	A1048501
CTS™ Immune Cell SR	A2596101
IL2 (Interleukin 2) CTS™ Recombinant Human Protein	CTP0023
GlutaMAX™ Supplement	35050061
CTS™ Dynabeads™ CD3/CD28	40203D
CTS™ DPBS without calcium chloride, without magnesium chloride	A1285601
CTS™ TrypLE™ Select Enzyme	A1285901
TrueGuide™ Synthetic gRNA	thermofisher.com/trueguide
TE, pH 8.0, RNase-free	AM9849
StemFlex™ Medium Kit	A3349401
CTS™ Xenon™ Genome Editing Buffer	A4998001
Neon™ Transfection System	MPK5000
Neon™ Transfection System 10 µL Kit	MPK1025, MPK1096
Neon™ Transfection System 100 µL Kit	MPK10025, MPK10096
Optional: GeneArt™ Genomic Cleavage Detection Kit	A24372

For Research Use or Non-Commercial Manufacturing of Cell-Based Products for Clinical Research. Caution: Not intended for direct administration into humans or animals.

ThermoFisher SCIENTIFIC

General transfection guidelines

- The efficiency with which mammalian cells are transfected with gRNA varies according to cell type and the delivery option used. See "Recommended delivery options".
- For gene editing (including gene knockout) editing efficiency is highest with a 1:1 molar ratio of gRNA to CTS™ TrueCut™ Cas9 Protein.
- For HDR knock-in editing, a 1.5:1 molar ratio of donor ssODN to gRNA or CTS™ TrueCut™ Cas9 Protein is recommend for highest knock-in efficiency. The donor can be added directly to RNPs (a premixed gRNA-Cas9 protein). If using a dsDNA donor, further optimization may be necessary to determine the appropriate donor amount, since the toxicity level is dependent on the length and format of the donor DNA and cell type.
- Use the TrueGuide™ positive controls (human AVVS1, CDK4, HPRT1, or mouse Rosa 26) and negative control gRNA (non-coding) to determine gRNA amount and transfection conditions that give the optimal gene editing efficiency with highest cell viability. The TrueGuide™ positive and negative sgRNA and crRNA controls are available separately from Thermo Fisher Scientific. For more information, see **thermofisher.com/trueguide**.

Recommended delivery options

Choosing the right delivery option is critical for transfection and gene editing efficiency.

Table 1 Recommended delivery options for CTS™ TrueCut™ Cas9 Protein

Cas9 format	Neon™ Transfection System	CTS™ Xenon™ Electroporation System
CTS™ TrueCut™ Cas9 Protein + gRNA	For high efficiency in difficult-to-transfect cell types or for HDR knock-in editing for research scale (up to 100 µL)	For large scale electroporation of primary and stem cells for cell therapy development and manufacturing

Prepare stock of TrueGuide™ Synthetic gRNA

If using TrueGuide™ Synthetic gRNA, resuspend the gRNA (sgRNA, crRNA, or tracrRNA) in 1X TE buffer to prepare 100 µM (100 pmol/µL) stock solutions.

1. Before opening, centrifuge each TrueGuide™ Synthetic gRNA tube at low speed (maximum RCF 4,000 × *g*) to collect the contents at the bottom of the tube, then remove the cap from the tube carefully.
2. Using a pipette and sterile tips, add the required volume of 1X TE buffer to prepare your designable concentration (20 µM to 100 µM) (20 to 100 pmol/µL) stock solutions.
3. Vortex the tube to resuspend the oligos. Briefly centrifuge to collect the contents at the bottom of the tube, then incubate at room temperature for 15–30 minutes to allow the gRNA oligos to dissolve.
4. Vortex the tube again to ensure that all the contents of the tube are resuspended, then briefly centrifuge to collect the contents at the bottom of the tube.
5. The gRNA working stocks can be used immediately or freeze at –20°C until needed for use.

Electroporation using the CTS™ Xenon™ Electroporation System

For electroporation of primary and stem cells for cell therapy development and manufacturing, see *CTS™ Xenon™ Electroporation System User Guide* (Pub. No. MAN0025488).

Electroporation using the Neon™ Transfection System

Protocols for the electroporation of T-cells and stem cells using the Neon™ Transfection System are provided as examples. Conditions may vary depending upon cell type and the downstream experimental procedure to be performed.

Isolate and activate T-cells (3 days before electroporation)

For optimal editing efficiency, it is recommended to culture the required number of T-cells for 3 days before performing electroporation using the Neon™ Transfection System. T-cells are activated and enriched from peripheral blood mononuclear cells (PBMC) isolated from healthy donors.

Prepare PBMC

1. Thaw enough vials of PBMC for the activation and enrichment of T-cells. Normally the same amount of T-cells can be obtained after 3-day activation from PBMC. For example, 50 million T-cells can be obtained from 50 million PBMC.

   For PBMC preparation from leukopak, we recommend the use of an automated systems such as the CTS™ Rotea™ Counterflow Centrifugation System. For instructions on how to prepare the cells, see *CTS™ Rotea™ Counterflow Centrifugation System User Guide* (Pub. No. MAN0018908)

Activate T-cells

2. Activate the T-cells (at 1×10^6 cells/mL) with CTS™ Dynabeads™ CD3/CD28 in CTS™ OpTmizer™ T-Cell Expansion SFM medium containing 100 U/mL CTS™ IL-2 Recombinant Human Protein, 6 mM GlutaMAX™ Supplement and 2% CTS™ Immune Cell SR.

   For complete protocol details, see *CTS™ Dynabeads™ CD3/CD28 User Guide* (Pub. No. MAN0008945).

Culture T-cells

3. Culture the T-cells in a humidified 37°C, 5% CO_2 incubator for 3 days before electroporation. For primary T-cells, this is typically double the amount used for adherent cells (e.g., $2–5 \times 10^5$ T-cells per well of a 24-well plate). See payload Table 2.

Prepare PSC culture (1–3 week before starting electroporation)

Prepare PSCs using growth medium of choice. We recommend the StemFlex™ Medium Kit. For complete protocol details, see *StemFlex™ Medium Kit User Guide* (Pub. No. MAN0016431).

PSCs culture typically requires 1–2 weeks before doing electroporation. The total cell number required for your experiment should be based on the Neon™ Electroporation System format of choice and the total number of samples.

Protocol for T-cells transfection using the Neon™ Transfection System

1 **Day 1:** Prepare plate with media

1.1. Add 0.5 mL of T-cell-specific growth medium into each well of the 24-well plate (if using 10 µL-Neon™ system) or 2 mL to each well of 6-well plate (if using 100 µL-Neon™ system).

1.2. Place the plate in the 37°C incubator to pre-warm.

2 **Day 1:** Prepare CRISPR Cas9/gRNA complex (and/or donor)

2.1. Mix the CTS™ TrueCut™ Cas9 Protein, gRNA, (and/or donor DNA) and Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer in a fresh, RNase-free microcentrifuge tube according to the payload table (Table 2). Mix well.

IMPORTANT!

- We recommend using CTS™ Xenon™ Genome Editing Buffer for improved performance, especially for HDR knock-in based applications.
- Always prepare reaction mixtures with reagent volumes for 1X extra reactions so there is sufficient volume to completely fill the Neon™ tip without bubbles, e.g. prepare 4X reactions if you do 3X Neon™ reactions per sample.
- Maintain CTS™ TrueCut™ Cas9 Protein:gRNA at a 1:1 molar ratio. Use high concentration CTS™ TrueCut™ Cas9 Protein and ensure that the total volume of the RNP complex (CTS™ TrueCut™ Cas9 Protein + gRNA) does not exceed 1/10th of the total reaction volume (e.g., 1 µL of Cas9 protein + gRNA in 10 µL total reaction volume).

Table 2 Payloads for Neon™ system

Neon™ system	10 µL Neon™ system	100 µL Neon™ system
Plates	Amount per well of 24-well plate	Amount per well of 6-well plate
Cell amount /well	2–5 × 10^5	2–5 × 10^6
Cas9 Protein	1.25 µg (7.5 pmol)	12.5 µg (75 pmol)
gRNA	0.24 µg (7.5 pmol)	2.4 µg (75 pmol)
Donor (75–100 bp)[1]	15 pmol	150 pmol
Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer	to 5 µL	to 50 µL

[1] ssOND donor is only required for knock-in (KI) editing. If using dsDNA, especially long dsDNA donor, optimization is required because the amount of donor DNA depends upon the size of the dsDNA.

2.2. Incubate the CRISPR-Cas9/gRNA complex in Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer at room temperature for 5–20 minutes.

3 **Day 1:** Prepare T-cells for electroporation

3.1. **Perform bead removal:** Transfer the appropriate number of cells into a 15-mL or 50-mL tubes for removal of magnetic beads. Gently mix T-cells by pipetting. De-bead by placing the tube in magnetic separator for 1–2 minutes. Transfer the supernatant containing the T-cells into a fresh tube.

3.2. Count the cell number and calculate the total number of cells required based on the payload (e.g. 2–5 ×10^5 T-cells per well of a 24-well plate), see Table 2. Centrifuge cells at 100–400 × *g* for 5 minutes at room temperature.

3.3. Wash the cells with CTS™ DPBS without calcium chloride, without magnesium chloride using the same volume as original cell volume, then pellet the cells by centrifugation at 100–400 × g for 5 minutes at room temperature.

3.4. **Resuspend the T-cells:** Aspirate the DPBS and resuspend the cell pellet in Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer at the desired concentration. For example, to use 200,000 cells/reaction, resuspend the cells at 40,000 cells/ µL, then use 5 µL (200,000 total cells) of the resuspended cells per reaction. Gently pipette the cells to obtain a single cell suspension.

4 **Day 1:** Add cells to CRISPR Cas9/gRNA complex

4.1. Pipette the T-cells (from "Day 1: Prepare T-cells for electroporation") up and down to resuspend any cells that might have settled at the bottom of the tube.

4.2. Add 5 µL (for 10 µL Neon™ system) or 50 µL (for 100 µL Neon™ system) of the cell suspension to the RNP (CRISPR-Cas9/gRNA complex and/or donor) from "Day 1: Prepare CRISPR Cas9/gRNA complex (and/or donor)".

Note: If preparing a 4x reaction to perform a 3x Neon™ electroporation, the total volume should be 40 µL for 10 µL-Neon™ or 400 µL for 100 µL-Neon™ system.

5 **Day 1:** Electroporate using cell-type specific Neon™ Transfection System conditions

5.1. Using the 10 µL or 100 µL Neon™ tip, aspirate the cell + RNP mix (CRISPR-Cas9/gRNA complex and/or donor), then electroporate using your cell type-specific Neon™ condition. For T-cell electroporation, we suggest using Program #24 (1300V / 10MS / 3 pulses).

IMPORTANT! Avoid creating bubbles that can hinder electroporation.

5.2. After electroporation, immediately transfer the contents of the Neon™ tip into one well of the plate containing pre-warmed growth medium as prepared in "Day 1: Prepare plate with media" on page 4.

5.3. Transfer the plate to a humidified 37°C, 5% CO_2 incubator, then incubate the cells fo 48 to 72 hours.

6 **Day 3–4:** Verify editing efficiency and proceed to downstream applications

After 48 to 72 hours incubation, use a small portion of T-cells for viability and following functional assays depending on your experiment (KI or KO) and sample number:

6.1. Flow cytometry if you have a proper antibody against your targeted gene for KI and KO (e.g. TCR). It is simple and quick assay to measure editing efficiency at function level.

6.2. NGS based sequencing assay for KI and KO, such as the Ion Torrent™ Targeted Amplicon-seq Validation (TAV). It is suitable for large sample number using 96-well format. The benefit using sequencing assay is to obtain the sequencing change information at genome level near the targeted region.

For more information on NGS assay, refer to "Additional CRISPR-Cas9 validation services" under "CRISPR Design to Validation Services" at **https://www.thermofisher.com/us/en/home/life-science/genome-editing/genome-engineering-services/crispr-design-validation-services.html**

6.3. GeneArt™ Genomic Cleavage Detection Kit (Cat. No. A24372) for KO assay. It is a T7 Endonuclease I (T7E1) mismatch assay and suitable for small sample number. For more information and detailed protocols, see the *GeneArt™ Genomic Cleavage Detection Kit User Guide* (Pub. No. MAN0009849), available for download at **thermofisher.com/GCDManual**.

Protocol for Pluripotent Stem Cells (PSCs) transfection using the Neon™ Transfection System

1 **Day 1:** Prepare plate with media

1.1. Add 0.5 mL of PSCs growth medium (e.gStemFlex™ Medium) into each well of the 24-well plate (if using 10 µL-Neon™ system) or 2 mL to each well of 6-well plate (if using 100 µL-Neon™ system).

1.2. Place the plate in the 37°C incubator to pre-warm.

2 **Day 1:** Prepare CRISPR-Cas9/gRNA complex

2.1. Mix the CTS™ TrueCut™ Cas9 Protein, gRNA, (and/or donor DNA) and Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer in a fresh, RNAse-free microcentrifuge tube according to the payload table (See Table 3). Mix well.

IMPORTANT!

- We recommend using CTS™ Xenon™ Genome Editing Buffer for improved performance, especially for HDR knock-in based applications.
- Always prepare reaction mixtures with reagent volumes for 1X extra reactions so there is sufficient volume to completely fill the Neon™ tip without bubbles, e.g. prepare 4X reactions if you do 3X Neon™ reactions per sample.
- Maintain CTS™ TrueCut™ Cas9 Protein:gRNA at a 1:1 molar ratio. Use high concentration CTS™ TrueCut™ Cas9 Protein and ensure that the total volume of the RNP complex (CTS™ TrueCut™ Cas9 Protein + gRNA) does not exceed 1/10th of the total reaction volume (e.g., 1 µL of Cas9 protein + gRNA in 10 µL total reaction volume).

Table 3 Payloads for Neon™ systems

Neon™ system	10 µL-Neon™ system	100 µL-Neon™ system
Plates	Amount per well of 24-well plate	Amount per well of 6-well plate
Cell amount/well	4–5 × 10^5	4–5 × 10^6
Cas9 Protein	1.25 µg (7.5 pmol)	12.5 µg (75 pmol)
gRNA	0.24 µg (7.5 pmol)	2.4 µg (75 pmol)
(Optional) ssOND Donor (75–100 bp)[1]	15 pmol	150 pmol
Resuspension Buffer R	to 5 µL	to 50 µL

[1] ssOND donor is only required for knock-in (KI) editing. If using dsDNA, especially long dsDNA donor, optimization is required because the amount depends upon the size of the dsDNA.

2.2. Incubate the CRISPR-Cas9/gRNA complex in Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer at room temperature for 5–20 minutes.

3 **Day 1:** Prepare PSCs for electroporation

3.1. Detach PSCs before electroporation with CTS™ TrypLE™ Select Enzyme. Rinse PSCs with proper amount (e.g. 2 mL per well if using 6-well plate) of DPBS, aspirate DPBS. Add proper amount (e.g. 1 mL per well if using 6-well plate) of CTS™ TrypLE™ Select Enzyme to cover surface of the well containing PSCs. Incubate for 2–3 minutes in a humidified 37°C, 5% CO_2 incubator.

3.2. Gently mix cells and transfer all PSCs to 15-ml or 50-mL tubes (depending on the amount of cells prepared). Centrifuge at 100–400 × *g* for 5 minutes at room temperature. Remove CTS™ TrypLE™ Select Enzyme.

3.3. Count the cell number and calculate the total cells required based on the table of payload above. Centrifuge enough cells at 100–400 × *g* for 5 minutes at room temperature.

3.4. Wash cells with DPBS, no calcium, no magnesium. Add proper amount of DPBS to the PSCs pellets and gently resuspend cells.

3.5. Resuspend PSCs in buffer of choice: Aspirate all DPBS (may need to use 20 µL pipette tip to remove all DPBS) and resuspend the cell pellet in Resuspension Buffer R or CTS™ Xenon™ Genome Editing Buffer at the desired concentration. For example, to use 400,000 cells/reaction, resuspend the cells at 800,000 cells/µL × 5 gives 400,000 cells/reaction of the resuspended cells per reaction. Gently pipette the cells to obtain a single cell suspension.

4 **Day 1:** Add cells to CRISPR-Cas9/gRNA complex

4.1. Pipette PSCs (from "Day 1: Prepare PSCs for electroporation" on page 6) up and down to resuspend any cells that might have settled at the bottom of the tube.

4.2. Add 5 µL (if using 10 µL-Neon™ system) or 50 µL (if using 100 µL- Neon™ system) of the cell suspension to the RNP (CRISPR-Cas9/gRNA complex and/or donor) from "Day 1: Prepare CRISPR Cas9/gRNA complex (and/or donor)" on page 4.

Note: If preparing a 4X reaction to perform a 3X Neon™ electroporation, the total volume should be 40 µL for 10 µL-Neon™ or 400 µL for 100 µL-Neon™.

5 **Day 1:** Electroporate using cell-type specific Neon™ condition

5.1. Using the 10 µL or 100 µL Neon™ system, aspirate the cell + RNP mix (CRISPR-Cas9/gRNA complex and/or donor), then electroporate using your cell type-specific Neon™ condition (see "Cell line specific electroporation conditions using the Neon™ Transfection System" on page 8). We suggest using the setting of 1300V / 30MS / 1 pulses.

IMPORTANT! Avoid creating bubbles that can hinder electroporation.

5.2. After electroporation, immediately transfer the contents of the Neon™ tip into one well of the plate containing pre-warmed growth medium (from "Day 1: Prepare plate with media" on page 6).

5.3. Transfer the plate to a humidified 37°C, 5% CO_2 incubator, then incubate the cells for one day.

6 **Day 2:** Change medium

6.1. Remove old medium and add 0.5 mL of pre-warmed growth medium (e.g. StemFlex™ Medium) into each well of the 24-well plate (if using 10 µL-Neon™ system) or 2 mL to each well of 6-well plate (if using 100 µL-Neon™ system).

6.2. Continue to grow cells for 4–5 days.

7 **Day 5:** Harvest cell and verify editing efficiency and proceed to downstream applications

7.1. Remove the old medium, rinse cells with DPBS and remove DPBS.

7.2. Detach cell by adding 0.1 mL CTS™ TrypLE™ Select Enzyme per well (if using 24-well plate) or 0.5 mL per well (if using 6-well plate). Incubate plate in a humidified 37°C, 5% CO_2 incubator for 5 minutes. Add 0.5 mL DPBS per well (if using 24-well plate) or 2 mL per well (if using 6-well plate). Mix and detach all cells. Transfer enough cells to a fresh tube for downstream assay.

7.3. Use a small portion of PSCs for viability and other following assays depending on your experiment (KI or KO) and sample number:

a. Flow cytometry if you have a proper antibody against your targeted gene for KI and KO (e.g. TCR). It is simple and quick assay to measure editing efficiency at function level.

b. NGS based sequencing assay for KI and KO, such as the Ion Torrent™ Targeted Amplicon-seq Validation (TAV). It is suitable for large sample number using 96-well format. The benefit using sequencing assay is to obtain the sequencing change information at genome level near the targeted region. For more information on NGS assay, refer to "Additional CRISPR-Cas9 validation services under "CRISPR Design to Validation Services" at Thermo Fisher Scientific website (**https://www.thermofisher.com/us/en/home/life-science/genome-editing/genome-engineering-services/crispr-design-validation-services.html**).

c. GeneArt™ Genomic Cleavage Detection Kit (Cat. No. A24372) fo KO assay. It is a T7 Endonuclease I (T7E1) mismatch assay and suitable for small sample number. For more information and detailed protocols, see *GeneArt™ Genomic Cleavage Detection Kit User Guide* (Pub. No. MAN0009849), available for download at **thermofisher.com/GCDManual**.

Release specification

Assay	Specification
Activity Assay, *in vitro*	≥ 90% *in vitro* of uncut reference DNA converted to cleavage products
Endotoxin by LAL	< 10.0 EU/mg
Sterility	No growth
Purity by HPLC-DAD	≥ 95.0%
Purity by SDS-PAGE	≥ 95.0%
Aggregation by SEC-HPLC	≤ 5.0%
Residual Host Protein by ELISA	< 10.0 ng/mL protein
Residual DNase	<LOQ DNaseALERT (LOQ = 2.04 pg/µL)
Residual RNase	<LOQ RNaseALERT (LOQ = 0.06 pg/µL)
Residual Host Genomic DNA by qPCR	<LOQ qPCR (LOQ = 0.3 pg/µL)
Residual Plasmid DNA by qPCR	<LOQ qPCR (LOQ = 0.96 copies/µL)
Mycoplasma by qPCR	<LOD qPCR (10 CFU/mL)

Cell line specific electroporation conditions using the Neon™ Transfection System

The following cell line specific conditions are provided as a starting point for transfecting cells with TrueGuide™ Synthetic gRNA and TrueCut™ Cas9 Protein using the Neon™ Transfection System 10 µL Kit. Further optimization of the electroporation or nucleofection conditions may be necessary for best results.

Cell type	Source	Media	Number of cells/10-µL reaction (× 10^3)	TrueCut™ Cas9 Protein/gRNA/ssODN (ng/pmoles/pmols)	Neon™ electroporation conditions[1]
Well format	—	—	24-well		
HEK293	Human embryonic kidney	DMEM	150	1250/7.5/11.25	1150 V/20 ms/2 pulses
U2OS	Human osteosarcoma	McCoy5A	150	1250/7.5/11.25	1400 V/15 ms/4 pulses
A549	Human epithelial lung carcinoma	DMEM	120	1250/7.5/11.25	1200 V/20 ms/4 pulses
THP1	Human peripheral blood monocyte leukemia	RPMI	200	2000/12/18	1700 V/20 ms/1 pulse (#5)
K562	Human leukemia bone marrow	RPMI	200	1250/7.5/11.25	1700 V/20 ms/1 pulse (#5)
iPSC	Human induced pluripotent stem cells	Essential 8™ Medium	80	1500/10/15	1200 V/20 ms/2 pulses (#14)
iPSC	Human induced pluripotent stem cells	StemFlex™ Medium	400,000 to 500,000	1500/10/15	1200 V/30 ms/1 pulse (#7)
Human primary T-cell	Healthy donor derived	OpTmizer™ + 2% human serum	200	1250/7.5/11.25	1600 V/10 ms/3 pulses (#24)
Jurkat T-cell	Human peripheral blood lymphocyte	RPMI	200	1250/7.5/11.25	1700 V/20 ms/1 pulse (#5)
HepG2	Human hepatocellular carcinoma	DMEM	120	1250/7.5/11.25	1300 V/30 ms/1 pulse (#8)
N2A	Mouse brain neuroblastoma	DMEM	100	1250/7.5/11.25	1400 V/30 ms/1 pulse (#9)

[1] Recommendations for the Neon™ electroporation settings are based on the culture conditions tested.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientific Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

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

Revision history: Pub. No. MAN0026510

Revision	Date	Description
B.0	25 August 2022	Updated shelf life to 24 months.
A.0	16 March 2022	New document for the use of CTS™ TrueCut™ Cas9 Protein.