TrueCut™ Cas9 Protein (Prototype)

Catalog Numbers A45220P, A45221P

Pub. No. MAN0019016 Rev. A.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

TrueCut™ Cas9 Protein (Prototype) 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 with aseptic filling, bioburden and endotoxin testing, and 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]
TrueCut™ Cas9 Protein (Prototype)	A45220P	2.5 mg	10 mg/mL	–20°C	NA
	A45221P	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] Product shelf life and performance claims have not been established.

Required material not supplied

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

Item	Cat. No
Dynabeads™ Untouched™ Human T Cells Kit	11344D
CTS™ OpTmizer™ T-Cell Expansion SFM	A1048501
CTS™ Immune Cell SR	A25961-01
IL2 (Interleukin 2) CTS™ Recombinant Human Protein	CTP0023
GlutaMAX™ Supplement	35050061
Dynabeads™ Human T-Activator CD3/CD28	11131D
DPBS, no calcium, no magnesium	14190144
TrueGuide™ Synthetic gRNA	thermofisher.com/trueguide
Neon™ Transfection System	MPK5000
Neon™ Transfection System 10 µL Kit	MPK1025, MPK1096
Optional: GeneArt™ Genomic Cleavage Detection Kit	A24372

For Research Use Only. Not for use in diagnostic procedures. Product is a prototype and performance characteristics of this product have not been established.

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Isolate and activate T-cells

1. Isolate the T-cells from peripheral blood mononuclear cells (PBMC) derived from healthy donors using the Dynabeads™ Untouched™ Human T Cells Kit.

2. Activate the T-cells (at 1 × 10^6 cells/mL) with Dynabeads™ Human T-Activator CD3/CD28 in CTS™ OpTmizer™ T-Cell Expansion SFM medium containing 100 U/mL IL2 (Interleukin 2) CTS™ Recombinant Human Protein, 6 mM GlutaMAX™ Supplement , and 2% CTS™ Immune Cell SR or human AB serum.

3. Culture the T-cells in a humidified 37°C, 5% CO_2 incubator for 3 days before electroporation.

Prepare CRISPR-Cas9/gRNA complex

1. Add 1 µg of TrueCut™ Cas9 Protein (Prototype) and 500 ng of gRNA to 5 µL of Resuspension Buffer R. Mix well gently.

   Note: Use high concentration Cas9 protein (10 µg/µL) and gRNA to keep the volume of Cas9/gRNA complex at less than 10% of total reaction volume (e.g., 1 µL of Cas9 protein + gRNA in 10 µL total reaction volume).

2. Incubate the Cas9/gRNA complex in Resuspension Buffer R at room temperature for 5–20 minutes.

Prepare T-cells for electroporation

1. To remove the Dynabeads™ Human T-Activator CD3/CD28 beads from the T-cells, place the tube on a magnetic rack for 1–2 minutes, then transfer the supernatant containing the T-cells to a new tube.

2. Count the T-cells, then collect 2 × 10^5 cells for each 10 µL Neon™ electroporation.

   Note: The optimal T-cell concentration for both 10-µL and 100-µL electroporations is 2 × 10^7–3 × 10^7 cells/mL.

3. Wash the T-cells once with DPBS, no calcium, no magnesium in 1.5-mL centrifuge tubes.

4. Resuspend the T-cells in 5 µL of Resuspension Buffer R, then gently mix with 6 µL of Cas9/gRNA complex.

   Note: We recommend preparing extra amount of cells needed to avoid pipette errors. For example, prepare 4 × 10^5 of T-cells in 10 µL Resuspension Buffer R, then transfer 5 µL of cells for one reaction.

5. *Optional*: For knock-in studies, add 0.5–1 µg of double-stranded or 10–100 pmol of single-stranded DNA into the mix, then electroporate.

Electroporate using the Neon™ Transfection System

1. Pipette 10 µL of the T-cells mixed with Cas9/gRNA complexes into the Neon™ 10-µL tip.

   IMPORTANT! Avoid creating bubbles, which can hinder electroporation.

2. Use program #24 (1600 V/10 ms/3 pulses) for electroporation.

   Programs #5, #16 and #23 also work well for T-cells.

3. Immediately transfer the electroporated cells into a 24-well plate containing 0.5 mL of pre-warmed culture medium described in “Isolate and activate T-cells”.

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

5. Verify the editing efficiency using flow cytometry or the GeneArt™ Genomic Cleavage Detection Kit.

Release specifications

Assay	Specification
Activity Assay, *in vitro*	≥ 90.0% *in vitro* of uncut reference DNA converted to cleavage products
Endotoxin by LAL	< 10.0 EU/mg
Bioburden	< 1 CFU/mL
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 = 1.07 pg/µL)
Residual RNase	<LOQ RNaseALERT v.2 (LOQ = 0.16 pg/µL)
Residual Host Genomic DNA by qPCR	<LOQ qPCR (LOQ = 0.02 pg/µL)
Residual Plasmid DNA by qPCR	<LOQ qPCR (LOQ = 0.01 pg/µL)
Mycoplasma by qPCR	<LOD qPCR (5 CFU/mL)

Limited product warranty

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