CRISPR/Cas9-mediated Genome Editing of Human Primary T-cells using the Neon™ Transfection System

Pub. No. MAN0017851 **Rev.** A.0

Note: 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**.

Overview

This protocol describes the isolation and activation conditions for human primary T-cells, as well as the procedure for the delivery of Cas9/gRNA (guide RNA) complexes via electroporation using the Neon™ Transfection System to T-cells.

Required materials not supplied

All materials are available through **thermofisher.com**.

Table 1 Materials required for T-cell isolation, activation, and CRISPR-Cas9 genome editing using the Neon™ Transfection System

Item	Catalog No.
Dynabeads™ Untouched™ Human T Cells Kit	11344D
CTS™ OpTmizer™ T Cell Expansion SFM	A1048501
CTS™ Immune Cell SR	A2596101
IL2 (Interleukin 2) CTS™ Recombinant Human Protein	CTP0023
GlutaMAX™ Supplement	35050061
Dynabeads™ Human T-Activator CD3/CD28	11131D
DPBS, no calcium, no magnesium	14190144
TrueCut™ Cas9 Protein v2	A36497
GeneArt™ Precision gRNA Synthesis Kit	A29377
Neon™ Transfection System	MPK5000
Neon™ Transfection System 10-µL Kit	MPK1025, MPK1096
(Optional) GeneArt™ Genomic Cleavage Detection Kit	A24372

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 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 CRIPSR-Cas9/gRNA complex

1. Generate the gRNA using the GeneArt™ Precision gRNA Synthesis Kit, or order TrueGuide™ Synthetic gRNA at **thermofisher.com/trueguide**.
2. Add 1 µg of TrueCut™ Cas9 Protein v2 and 250 ng of gRNA to 5 µL of Resuspension Buffer R. Mix well gently.

   Note: Use high concentration Cas9 protein (5 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).

   Note: The amounts of reaction components are for a single electroporation using the 10-µL Neon™ tip. For 100 µL, you can linearly scale up the payload amount.
3. 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 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 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 50–100 pmol of single-stranded DNA (Shumman K, et al., 2015) into the mix, then electroporate. Alternatively, use AAV6 to deliver donor DNA after Cas9/gRNA complex delivery by the Neon™ Transfection System.

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.

   Note: Programs #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 hours.
5. Verify the editing efficiency using flow cytometry or the GeneArt™ Genomic Cleavage Detection Kit.

Reference

Shumman K, Lin S, Boyer E, et al. (2015) Generation of knock-in primary human T cells using Cas9 ribonucleoproteins. *Proc Natl Acad Sci USA* 112:10437–10442. doi: 10.1073/pnas.1512503112.

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 found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Manufacturer: Life Technologies Corporation | 5791 Van Allen Way | Carlsbad, CA 92008

thermofisher.com/support | thermofisher.com/askaquestion

thermofisher.com

01 May 2018