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Produktinformation

- Forschungsprodukte & Biochemikalien
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- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The TCR Knockout Electroporation Kit is suitable for Jurkat cell line and primary T cell engineering using electroporation. The kit contains both the Cas9 enzyme (*Streptococcus pyogenes*) and the gRNAs targeting TRAC (T-Cell Receptor Alpha Constant) and TRBC (targets both T Cell Receptor Beta Constant 1 and T Cell Receptor Beta Constant 2). This kit is sufficient to engineer up to 5 million primary T cells.

Background

The TCR (T Cell Receptor) is found on the surface of T cells and is responsible for recognizing antigens bound to MHC (Major Histocompatibility Complex) molecules. Stimulation of the TCR results in activation of downstream NFAT (Nuclear factor of activated T-cells) signaling. NFAT is a family of transcription factors that has an important function in immune responses, for example by inducing the expression of various cytokines (such as interleukin-2 to 4, and TNF-alpha (tumor necrosis factor alpha)) in T cells. The TCR consists of a heterodimer of two different protein chains, of which the alpha (α) and beta (β) chains are the predominant chains. Knocking out the TCR molecule in T cells ensures the specificity of engineered TCR or CAR-T cells that target tumor-associated antigens and is a critical step in the development of adoptive cell therapies as the endogenous TCR could interfere by recognizing other antigens not intended for targeting. TCR-KO T cells rely exclusively on the engineered receptor for antigen recognition, thereby improving the specificity and potency of the therapy.

Application

- Knockout of TCR α/β in cells of interest.

Supplied Materials

Catalog #	Name	Amount	Storage
	TRAC gRNA	10 µl	-20°C
	TRBC gRNA	10 µl	-20°C
	Cas9 enzyme (*Streptococcus pyogenes*)	10 µl	-20°C

Storage Conditions

Components are shipped in dry ice and stored at -20°C freezer for long term storage. The components maintain their stability and performance after 5 freeze-thaw cycles.

Materials Required but Not Supplied

These materials are not supplied with the kit but maybe required for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this kit and are highly recommended for the best results.

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Name	Ordering Information
TCellM™	BPS Bioscience #78753
Normal Human Peripheral Blood Mononuclear Cells, Frozen	BPS Bioscience #79059
Human Interleukin-2 Recombinant	BPS Bioscience #90184
EasySep™ Human CD4+ T Cell Isolation Kit	Stemcell technologies #17952
EasySep™ Human CD8+ T Cell Isolation Kit	Stemcell technologies #17953
Human CD3/CD28/CD2 T Cell Activator APC anti-human TCR α/β Antibody	Stemcell technologies #10970 BioLegend #306718
Neon NxT Electroporation System (or other electroporation system)	NEON1SK

Media Required for T cell Culture

T Cell Medium: TCellM™ (BPS Bioscience #78753) supplemented with 10 ng/ml Human Interleukin-2 Recombinant (BPS Bioscience #90184).

Assay Protocol

- The following protocol was used to knock out the TCR in $CD4^+$ and $CD8^+$ primary T cells using the TCR Knockout Electroporation kit, and it is a general guideline only.
- The assay conditions (concentration of gRNAs and Cas9 enzyme, and time of assay) should be optimized according to the cell type, donor, and the assay requirements.

Day 0:

1. Isolate $CD4^+$ T cells and $CD8^+$ T cells from previously frozen human PBMC by negative selection, according to the manufacturer's instructions.

2. Mix $CD4^+$ T cells and $CD8^+$ T cells at a 1:1 ratio.

3. Culture cells in T Cell Medium at 1 x 10^6 cells/ml, at 37°C with 5% CO_2 overnight.

Day 1:

1. Activate T cells with the Human CD3/CD28/CD2 T cell Activator reagent, following the vendor's recommendation, and incubate at 37°C with 5% CO_2 for 24 - 48 hours.

Day 3:

1. Prepare the Cas9 RNP Complex in Resuspension Buffer R (provided with Neon NxT Electroporation System) as shown in the table below.

   Note: Always include extra volume to avoiding pipetting errors and creation of bubbles.

Component	**Amount**
TRAC gRNA	0.5 µl
TRBC gRNA	0.5 µl
Cas9 enzyme	0.5 µl
Resuspension Buffer R	3.5 µl
Total	**5 µl**

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2. Incubate the mixture at Room Temperature (RT) for 15 minutes.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and wash the cells with PBS.

4. Spin down the cells again, and resuspend the cell pellet in Resuspension Buffer R at a density of 4 x 10^7cells/ml.

5. For each reaction, add 5 µl of the cell suspension to 5 µl of the Cas9 RNP Complex in Resuspension Buffer R (from step 1).

6. Using the 10 µl Neon™ tip, pipet 10 µl of cells/Cas9 RNP complexes, and electroporate using an optimized protocol for primary T cells (1,600V, 10 ms, 3 pulses).

Note: Avoid creating bubbles, which can affect the electroporation efficiency.

7. After electroporation, immediately transfer the contents of the Neon tip into one well of the 6-well culture plate containing 2 ml of prewarmed T Cell Medium.

8. Incubate the cells at 37°C for 2-3 days.

Day 5-6:

1. Analyze the TCR Knockout efficiency by flow cytometry, or other method of interest.

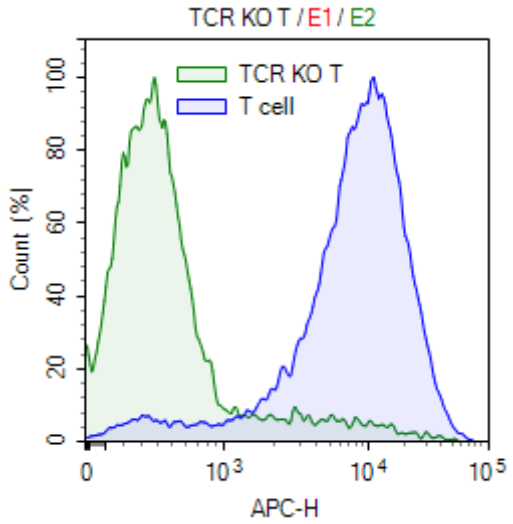

Figure 1: TCR knockout in CD4⁺ and CD8⁺ T cells by flow cytometry.
Approximately 250,000 CD4⁺ and CD8⁺ activated T cells were mixed with Cas9 RNP Complexes and electroporated using the Neon NxT Electroporation System. 72 hours after electroporation, TCR expression was analyzed by flow cytometry using APC anti-human TCR α/β Antibody (Biolegend #306717). Non-electroporated T cells were used as control (blue). T cells where TCR was knockout are shown in green. The y axis represents the % of cells, while the x axis represents the fluorophore intensity.

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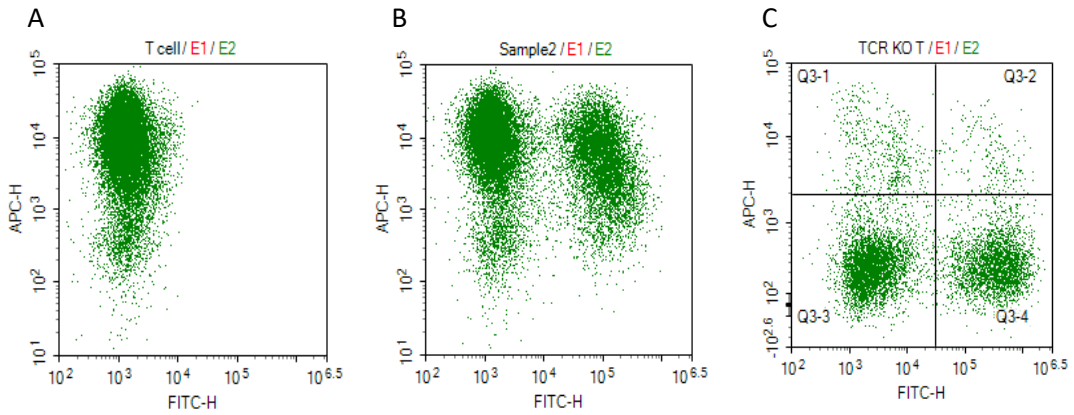

Figure 2: TCR Knockout in CD19 CAR-T cells.
$CD4^+$ and $CD8^+$ activated T cells were transduced with Anti-CD19 CAR Lentivirus (CD19 ScFv-CD8-4-1BB-CD3ζ; SIN Vector) (#78601). 72 hours after transduction, approximately 250,000 transduced cells were mixed with Cas9 RNP complexes and electroporated using the Neon NxT Electroporation System. 72 hours after electroporation, CD19 CAR expression was analyzed by flow cytometry using FITC-Labeled Monoclonal Anti-FMC63 Antibody, Mouse IgG1 (AcroBiosystems #FM3-FY45G0), and TCR expression was analyzed using APC anti-human TCR α/β Antibody (Biolegend #306717). Panel A: control T cells; Panel B: T cells transduced with CD19 CAR lentivirus; Panel C: T cells transduced with CD19 CAR lentivirus and electroporated using the TCR Knockout Electroporation kit.

Gene Target	sgRNA Sequence
TRAC	TGTGCTAGACATGAGGTCTA
TRBC1	GCAGTATCTGGAGTCATTGA

Figure 3: sgRNA sequences of the sgRNAs included in the TCR Knockout Electroporation Kit.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Notes

The CRISPR/CAS9 technology is covered under numerous patents, including U.S. Patent Nos. 8,697,359 and 8,771,945, as well as corresponding foreign patents applications, and patent rights.

Related Products

Products	*Catalog #*	*Size*
TCR Knockout NFAT-Luciferase Reporter Jurkat cell Line	78556	2 vials
TCR CRISPR/Cas9 Lentivirus (Integrating)	78055	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2

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