Description
TCR Knockout Jurkat Cell Line is a Jurkat cell line generated by CRISPR/Cas9 genome editing to remove the TRAC (T-Cell Receptor Alpha Constant) and TRBC1 (T-Cell Receptor Beta Constant 1) domains of the TCRα and β chains.

Background
The T-Cell Receptor (TCR) is found on the surface of T cells and is responsible for recognizing antigens bound to MHC (Major Histocompatibility Complex) molecules. Engagement of the TCR initiates downstream NFAT (nuclear factor of activator T cells) signaling resulting in T cell activation. The TCR consists of a heterodimer of two different protein chains, of which the alpha (α) and beta (β) chains are the predominant chains. CRISPR/Cas9 genome editing was utilized to remove the TRAC (T-Cell Receptor Alpha Constant) and TRBC1 (T-Cell Receptor Beta Constant 1) regions of the α and β chains, resulting in a loss of TCR expression.

Application
Use as a control when generating or characterizing CAR-T Cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience #79796)

Parental Cell Line
Jurkat (clone E6-1), human T lymphoblast, suspension

Mycoplasma Testing
The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied
These materials are not supplied with the cell line but are necessary for cell culture and cellular assays. BPS Bioscience's reagents are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section below.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184

Storage Conditions
Cells are shipped in dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations
For best results, the use of validated and optimized media from BPS Bioscience *is highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do not contain selective antibiotics.
Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Cell Thawing

1. Retrieve a cell vial from liquid nitrogen storage. Keep on dry ice until ready to thaw.

2. When ready to thaw, swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. Once cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire content of the vial to an empty 50 ml conical tube.

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

3. Using a 10 ml serological pipette, slowly add 10 ml of pre-warmed Thaw Medium 2 to the conical tube containing the cells. Thaw Medium 2 should be added dropwise while gently rocking the conical tube to permit gentle mixing and avoid osmotic shock.

4. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2.

5. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

6. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

7. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Thaw Medium 2.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml in Thaw Medium 2. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

Note: It is recommended to expand the cells and freeze at least 10 vials at an early passage for future use.

Validation Data

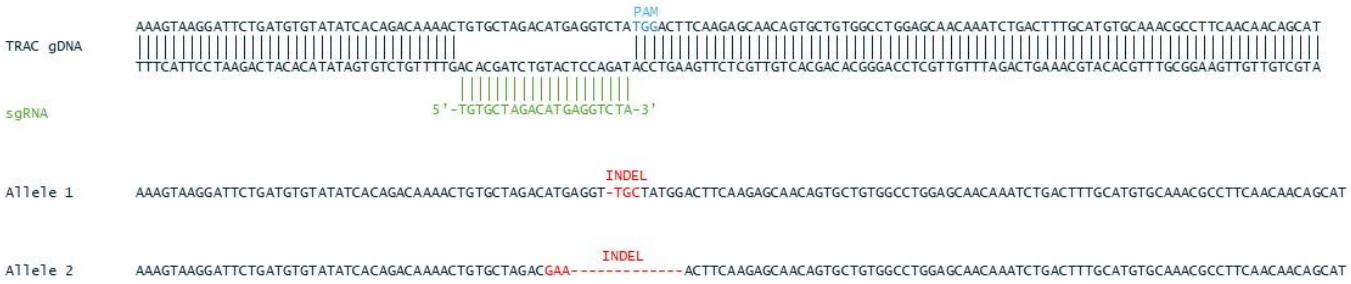

Figure 1. Genomic sequencing of TRAC in the TCR Knockout Jurkat Cell Line.
Genomic DNA from TCR Knockout Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green, and the Indels (Insertions / Deletions) in the two TRAC alleles are indicated in red.

Figure 2. Genomic sequencing of TRBC1 in the TCR Knockout Jurkat Cell Line.
Genomic DNA from TCR Knockout Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green, and the Indels (Insertions / Deletions) in the two TRBC1 alleles are indicated in red.

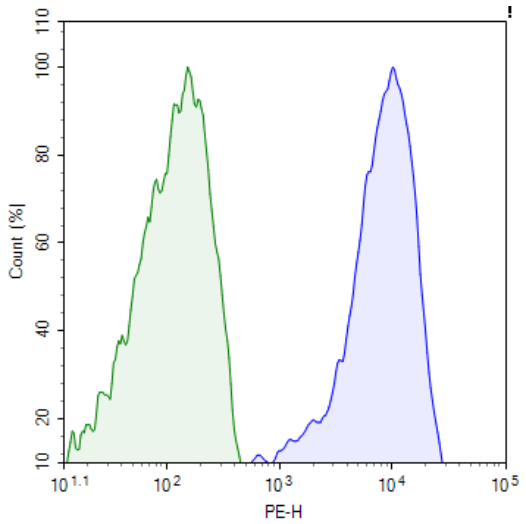

Figure 3. Expression of TCR in the TCR Knockout Jurkat Cell Line.
TCR Knockout Jurkat cells (green) or parental Jurkat cells (blue) were stained with PE anti-human α/β T Cell Receptor Antibody (BioLegend #306707) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

Sequence

Human mRNA for T-Cell Receptor Alpha Chain (GenBank Accession #X02592.1), with the sgRNA targeting sequence underlined:

atgctcctgctgctcgtcccagtgctcgaggtgatttttaccctgggaggaaccagagcccagtcggtgacccagcttggcagccacgtctctgtctctgaaggagc
cctggttctgctgaggtgcaactactcatcgtctgttccaccatatctcttctggtatgtgcaataccccaaccaaggactccagcttctcctgaagtacacatcagcg
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acctgtcagtgattgggttccg aatcctcctcctgaaagtggccgggtttaatctgctcatgacgctgcggctgtggtccagctga

Human mRNA for T-Cell Receptor Beta Chain (GenBank Accession #NG_001333), with the sgRNA targeting sequence underlined:

Atgggctgcaggctgctctgctgtgcggttctctgtctcctgggagcagttcccatagacactgaagttacccagacaccaaaacacctggtcatgggaatgacaa
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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.

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Troubleshooting Guide

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

Related Products

Products	*Catalog #*	*Size*
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78556	2 vials
TCR/B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78557	2 vials
TCR Activator Raji Cell Line	60556	2 vials

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