Description

TCR/B2M Knockout Jurkat Cell Line is a Jurkat cell line with a double knockout of TCR (T Cell Receptor) and B2M (Beta-2-Microglobulin). First, the TRAC (T-Cell Receptor Alpha Constant) and the TRBC1 (T-Cell Receptor Beta Constant 1) domains of the TCRα/β chains were genetically removed by CRISPR/Cas9 genome editing from Jurkat cells to generate the TCR Knockout Jurkat cell Line (BPS Bioscience #78539). These TCR knockout cells were then used to generate a new cell line in which B2M was also genetically removed by CRISPR/Cas9 genome editing.

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. Stimulation of the TCR triggers a signaling cascade that leads to the activation of transcription factors involved in the upregulation and secretion of cytokines, T cell proliferation, and cell differentiation into effector and memory cells. TCR-activated transcription factors include AP-1 (Activator Protein 1), NF-κB (Nuclear Factor Kappa-light-chain-enhancer of activated B cells) and NFAT (Nuclear Factor of Activated T-cells).

Beta-2-Microglobulin is a required component of Major Histocompatibility Complex (MHC) class 1 molecules, which present peptide fragments from within the cell to cytotoxic T cells as part of the adaptive immune system. B2M plays an essential role both in governing MHC class I molecule stability and in promoting antigen binding by presenting the antigen to CD3/TCR complex of $CD8^+$ T cells.

Knockout of both TCR and B2M will support the manufacture of universal CAR-T cells. Knockout of TCR or B2M prevents the elimination of allogeneic T cells that express foreign HLA-I molecules, thereby enabling the generation of CAR-T cells from allogeneic healthy donors T cells *in vivo*.

Application(s)

Use as control when developing improved universal CAR-T or other effector 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℃ 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, bu 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.

A. Validation Data

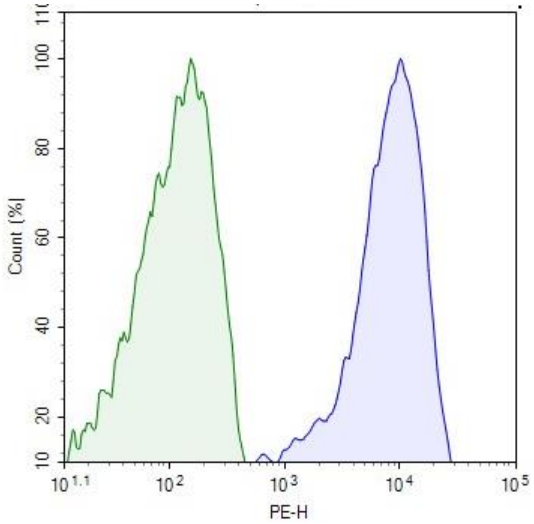

Figure 1. Expression of TCR in the TCR/B2M Knockout Jurkat Cell Line by flow cytometry.
TCR/B2M Knockout Jurkat cells (green) or parental Jurkat cells (blue) were stained with PE anti-human TCR α/β T Cell Receptor Antibody (BioLegend #306707) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates PE intensity.

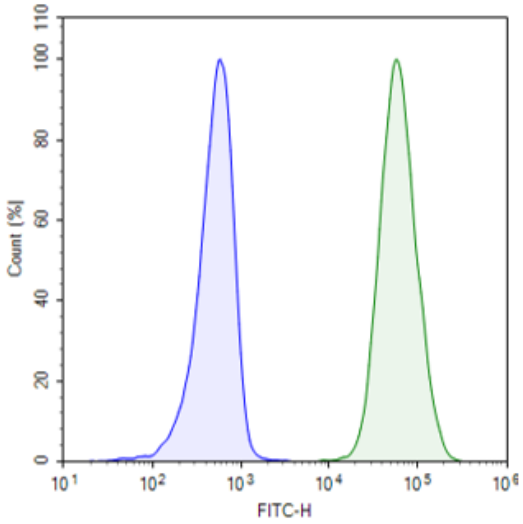

Figure 2. Expression of B2M in the TCR/B2M Knockout Jurkat Cell Line by flow cytometry.
TCR/B2M Knockout Jurkat cells (blue) or parental Jurkat cells (green) were stained with Alexa 488® Mouse anti-Human HLA-ABC antibody (BD Pharmingen #560169) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates FITC intensity.

Sequences

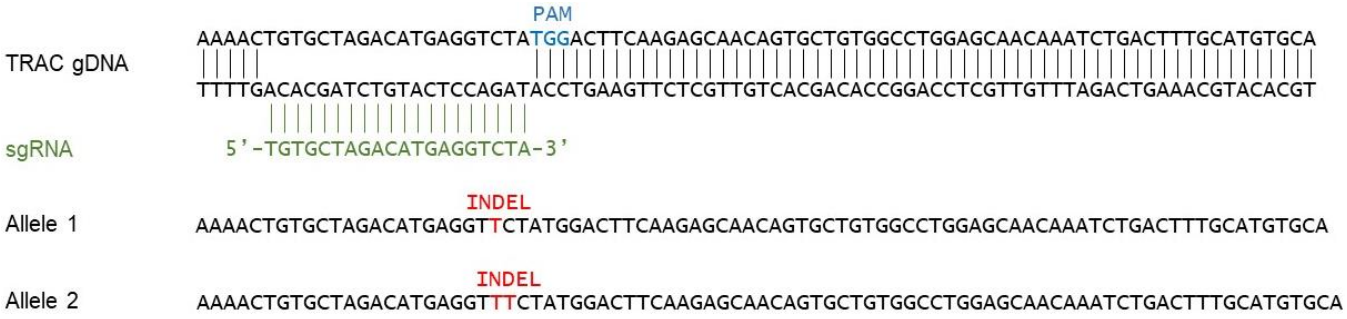

Figure 3. Genomic sequencing of TRAC in the TCR/B2M Knockout Jurkat Cell Line.
Genomic DNA from TCR/B2M Knockout Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green, and the Indels (Insertions/Deletions) in the two TRAC alleles are indicated in red.

Figure 4. Genomic sequencing of TRBC1 in the TCR/B2M Knockout Jurkat Cell Line.
Genomic DNA from TCR/B2M 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.

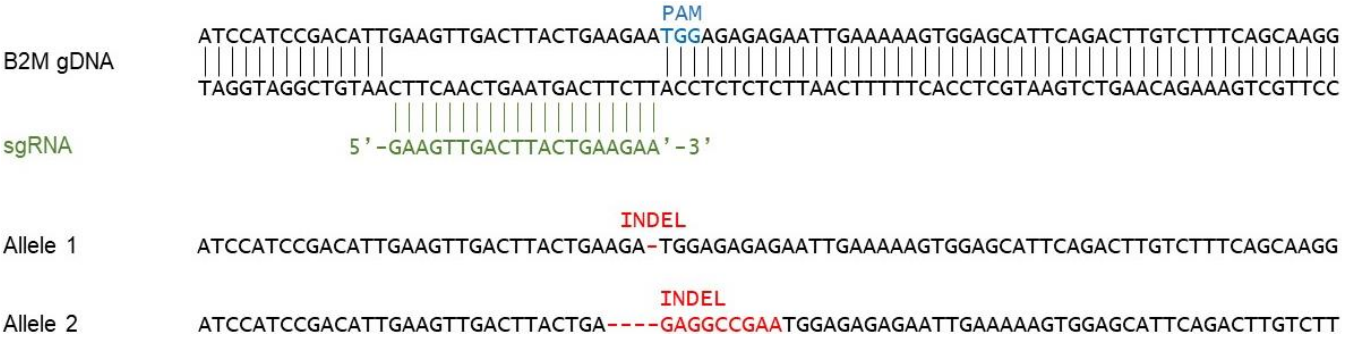

Figure 5. Genomic sequencing of B2M in the TCR/B2M Knockout Jurkat Cell Line.
Genomic DNA from TCR/B2M Knockout Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green, and the Indels (Insertions/Deletions) in the two B2M alleles are indicated in red.

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

atgctcctgctgctcgtcccagtgctcgaggtgatttttaccctgggaggaaccagagcccagtcggtgacccagcttggcagccacgtctctgtctctgaaggagc
cctggttctgctgaggtgcaactactcatcgtctgttccaccatatctcttctggtatgtgcaataccccaaccaaggactccagcttctcctgaagtacacatcagcg
gccaccctggttaaaggcatcaacggttttgaggctgaatttaagaagagtgaaacctccttccacctgacgaaaccctcagcccatatgagcgacgcggctgagt
acttctgtgctgtgagtgatctcgaaccgaacagcagtgcttccaagataatctttggatcagggaccagactcagcatccggccaaatatccagaaccctgaccc
tgccgtgtaccagctgagagactctaaatccagtgacaagtctgtctgcctattcaccgattttgattctcaaacaaatgtgtcacaaagtaaggattctgatgtgta
tatcacagacaaaactgtgctagacatgaggtctatggacttcaagagcaacagtgctgtggcctggagcaacaaatctgactttgcatgtgcaaacgccttcaac
aacagcattattccagaagacaccttcttccccagcccagaaagttcctgtgatgtcaagctggtcgagaaaagctttgaaacagatacgaacctaaactttcaaa
acctgtcagtgattgggttccgaatcctcctcctgaaagtggccgggtttaatctgctcatgacgctgcggctgtggtccagctga

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

atgggctgcaggctgctctgctgtgcggttctctgtctcctgggagcagttcccatagacactgaagttacccagacaccaaaacacctggtcatgggaatgacaa
ataagaagtctttgaaatgtgaacaacatatggggcacagggctatgtattggtacaagcagaaagctaagaagccaccggagctcatgtttgtctacagctatg
agaaactctctataaatgaaagtgtgccaagtcgcttctcacctgaatgccccaacagctctctcttaaaccttcacctacacgccctgcagccagaagactcagcc
ctgtatctctgcgccagcagccccctcgggagggagggactgaacactgaagctttctttggacaaggcaccagactcacagttgtagaggacctgaacaaggtg
ttcccacccgaggtcgctgtgtttgagccatcagaagcagagatctcccacacccaaaaggccacactggtgtgcctggccacaggcttcttccccgaccacgtgg
agctgagctggtgggtgaatgggaaggaggtgcacagtggggtcagcacggacccgcagcccctcaaggagcagcccgcccctcaatgactccagatactgcctg
agcagccgcctgagggtctcggccaccttctggcagaacccccgcaaccacttccgctgtcaagtccagttctacgggctctcggagaatgacgagtggacccag
gatagggccaaacccgtcacccagatcgtcagcgccgaggcctggggtagagcagactgtggctttacctcggtgtcctaccagcaaggggtcctgtctgccacc
atcctctatgagatcctgctagggaaggccaccctgtatgctgtgctggtcagcgcccttgtgttgatggccatggtcaagagaaaggatttctga

Human mRNA for Beta-2-microglobulin (NCBI Reference Sequence #NM_004048.4), with the sgRNA targeting sequence underlined:

atgtctcgctccgtggccttagctgtgctcgcgctactctctctttctggcctggaggctatccagcgtactccaaagattcaggtttactcacgtcatccagcagaga
atggaaagtcaaatttcctgaattgctatgtgtctgggtttcatccatccgacattgaagttgacttactgaagaatggagagagaattgaaaaagtggagcattca
gacttgtctttcagcaaggactggtctttctatctcttgtactacactgaattcacccccactgaaaaagatgagtatgcctgccgtgtgaaccatgtgactttgtcac
agcccaagatagttaagtgggatcgagacatgtaa

License Disclosure

Visit bpsbioscience.com/license for the label license and other key information about this product.

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 Jurkat Cell Line	78539	2 vials
TCR Activator Raji Cell Line	60556	2 vials
TCR Activator Lentivirus (CMV Promoter/Puromycin) or (EF1a Promoter/Puromycin) or (EF1a Promoter/Hygromycin)	79894	500 µl x 2
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
FcRn (FCGRT/B2M) Blocker	101468	50 µg/100 µg
B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78341	500 µl x 2

Version 112823