Description

This cell line is 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 NFAT Luciferase Reporter Jurkat cells to generate the TCR Knockout NFAT Luciferase Reporter Jurkat cell Line (BPS Bioscience #78556). These TCR knockout cells were used to generate a new cell line in which B2M was also genetically removed by CRISPR/Cas9 genome editing.

Expression of the firefly luciferase gene is driven by NFAT response elements located upstream of the minimal TATA promoter. Activation of the NFAT signaling pathway in these cells can be monitored by measuring luciferase activity.

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 signaling. NFAT (Nuclear factor of activated T-cells) 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) in T cells. NFAT is regulated by Ca^{2+} and the Ca^{2+}/calmodulin-dependent serine phosphatase, calcineurin.

Beta-2-Microglobulin is a required component of MHC class 1 molecules, which present peptide fragments from within the cell to cytotoxic T cells as part of the adaptive immune system. The protein forms amyloid fibrils in some pathological conditions. B2M plays an essential role both in governing MHC class I molecule stability and in promoting antigen binding and presenting the antigen to CD3/TCR complex of CD8+ T cells.

The knockout of both TCR and B2M will support the manufacture of universal CAR-T cells. The ablation of B2M or TCR prevents the elimination of allogeneic T cells that express foreign HLA-I molecules, and thereby enables the generation of CAR-T cells from allogeneic healthy donors T cells with higher persistence *in vivo.*

Application(s)

Develop improved universal CAR-T or other effector cells.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains 2 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
Growth Medium 2B	BPS Bioscience #79530

Materials Required for Cellular Assay

Name	Ordering Information
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
NFAT Luciferase Jurkat Recombinant Cell Line	BPS Bioscience #60621
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white, clear-bottom plate	
Luminometer	

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. Other preparations or formulations of media may result in suboptimal performance.

Note: Thaw Media do *not* contain selective antibiotics. However, Growth Media *do* contain selective antibiotics, which are used for maintaining the presence of the transfected gene(s) over passages. Cells should be grown at 37℃ with 5% CO_2. BPS Bioscience's cell lines are stable for at least 15 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

Growth Medium 2B (BPS Bioscience #79530):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin

Media Required for Functional Cellular Assay

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

Cell Culture Protocol

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2 **(no Geneticin)**.
   Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. 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 **(no Geneticin).**

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

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2 **(no Geneticin)**, and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6. At first passage and subsequent passages, use Growth Medium 2B **(contains Geneticin).**

Cell Passage

Dilute the cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml of Growth Medium 2B **(contains Geneticin)**. The sub-cultivation ratio is approximately 1:6 to 1:8 weekly or twice per week, so cells are maintained 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell aliquots into cryogenic vials. 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 storage.

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

Validation Data

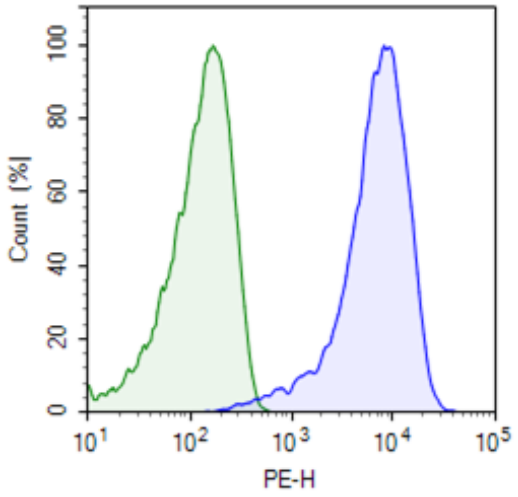

Figure 1: Analysis of TCRα/β Expression in TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells.
TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells were incubated with a PE-labeled anti-human TCRα/β antibody (BioLegend #306707) and analyzed by flow cytometry. Parental NFAT-Luciferase Jurkat cells are shown in blue, compared to the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells (green). Y-axis is the % cell number. X-axis is the intensity of PE.

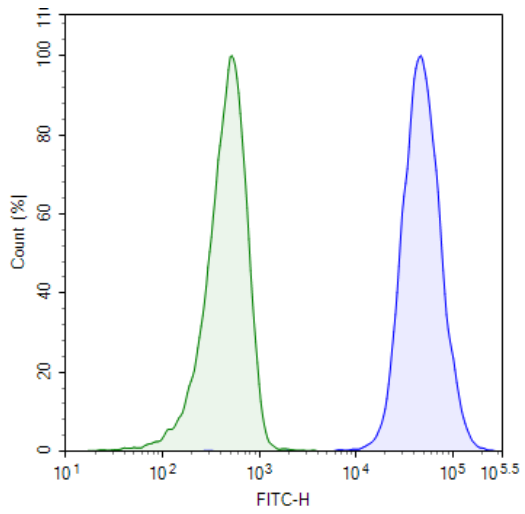

Figure 2: Analysis of B2M Expression in TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells.
TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells were incubated with a 488-labeled anti-human HLA-ABC antibody (BD Pharmingen #560169) and analyzed by flow cytometry. Parental NFAT-Luciferase Jurkat cells are shown in blue, compared to the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells (green). Y-axis is the % cell number. X-axis is the intensity of FITC.

Sequences

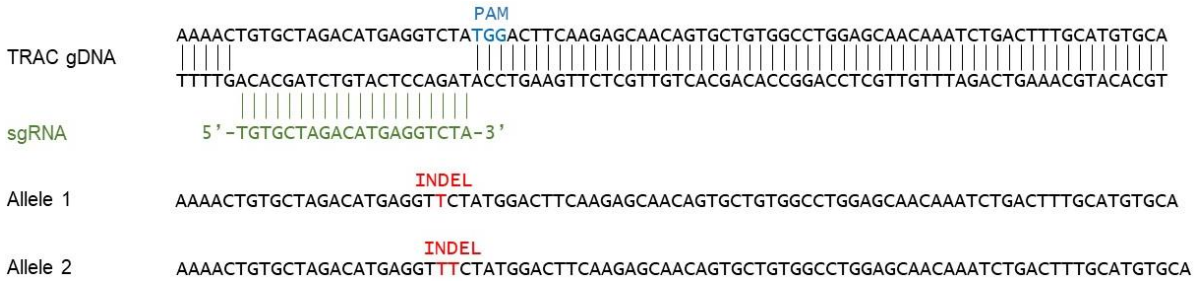

Figure 3. Genomic Sequencing of TRAC in the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat Cell Line.
The genomic DNA from the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in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

Figure 4. Genomic Sequencing of TRBC1 in the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells.
The genomic DNA from the TCR/B2M Knockout NFAT-Luciferase Reporter 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 indicated in red.

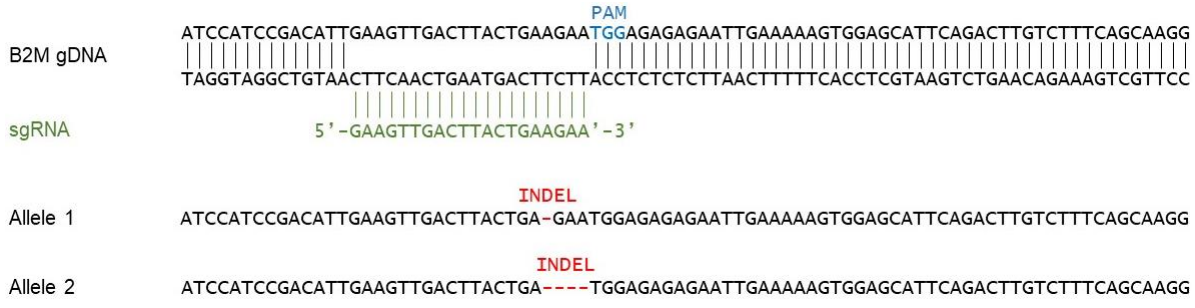

Figure 5. Genomic Sequencing of B2M in the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells.
The genomic DNA from the TCR/B2M Knockout NFAT-Luciferase Reporter Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green,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

Assay Protocol to test NFAT Luciferase Reporter Activity stimulated by anti-CD3 agonist
Perform the experiment at least in triplicates.

1. Centrifuge TCR/B2M Knockout NFAT Luciferase Reporter Jurkat cells and positive control NFAT Luciferase Reporter Jurkat cells and resuspend in Thaw Medium 2 at a density of 5 x 10^5 cells/ml (50 µl/well, triplicates).

2. Prepare dilutions of the anti-CD3 agonist antibody (e.g., 10 µg/ml) in 50 µl/well of the Thaw Medium, at concentrations 2-fold higher than the desired final concentrations. Mix 50 µl of antibody with 50 µl of the TCR/B2M Knockout NFAT Luciferase Reporter or control NFAT Luciferase Reporter Jurkat cells. Plate 100 µl per well into a white clear-bottom 96-well plate in triplicates. The final cell density of the NFAT Luciferase Reporter Jurkat cells is 25,000 cells per well.

3. Add 50 µl of Thaw Medium 2 to 50 µl of unstimulated control cells.

4. Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining background luminescence).

5. Incubate the plates at 37˚C in a CO_2 incubator for 5-6 hours.

6. After the incubation, measure luciferase activity using the One-Step Luciferase Assay System: Add 100 µl of ONE-Step Luciferase Reagent per well and rock gently at room temperature for ~15 minutes. Measure the luminescence using a luminometer.

7. Data Analysis: Subtract the average background luminescence of the cell-free control wells from the luminescence reading of all wells.

8. The fold induction of NFAT luciferase reporter expression is the average background-subtracted luminescence of antibody-treated well divided by the average background-subtracted luminescence of unstimulated control wells.

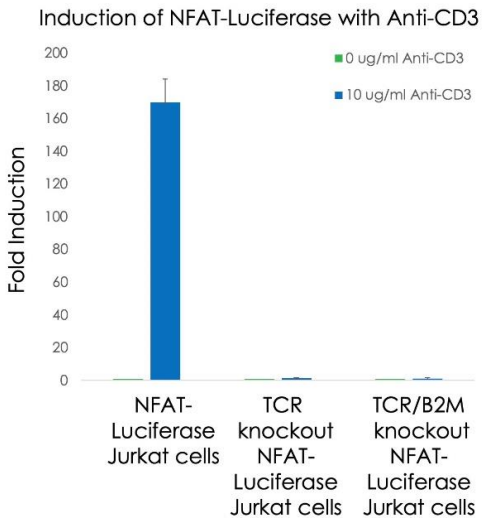

Figure 6. Stimulation of NFAT Luciferase activity with anti-CD3 Agonist Antibody.
Parental NFAT Luciferase Jurkat cells (positive control), TCR knockout or TCR/B2M knockout NFAT Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a clear-bottom white 96-well plate. Each cell line was incubated with either 0 or 10 µg/ml anti-CD3 for 5 hours at 37°C. After incubation, ONE-Step Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure NFAT activity. Background signal (no cells) was subtracted from all other values. The graph shows that NFAT signaling was not activated by the anti-CD3 agonist antibody in B2M knock-out cells or in TCR/B2M knockout cells.

Functional assay: stimulation of NFAT-Luciferase activity using Ionomycin/PMA.
The assay should be performed in triplicates.

1. Seed TCR Knockout NFAT-Luciferase Jurkat cells at a density of 25,000 cells per well into white clear-bottom 96-well microplate in 50 µl of Thaw Medium 2.

2. Prepare an intermediate solution of Ionomycin and PMA by diluting it into Thaw Medium 2 at a concentration that is 2-fold higher than the desired final concentration. Add 50 µl of the diluted compound to each well (the final volume is 100 µl).

3. Add 50 µl of Thaw Medium 2 to 50 µl of unstimulated control cells.

4. Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining the background luminescence).

5. Incubate at 37°C with 5% CO_2 for 16-24 hours.

6. Measure the luciferase activity using the ONE-Step™ Luciferase Assay System. Add 100 µl/well of ONE-Step™ Luciferase Reagent per well and rock gently at room temperature for ~15 minutes. Measure the luminescence using a luminometer.

7. Data Analysis: Subtract the average background luminescence of the cell-free control wells from the luminescence reading of all wells. The fold induction of NFAT luciferase reporter expression is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of untreated control wells.

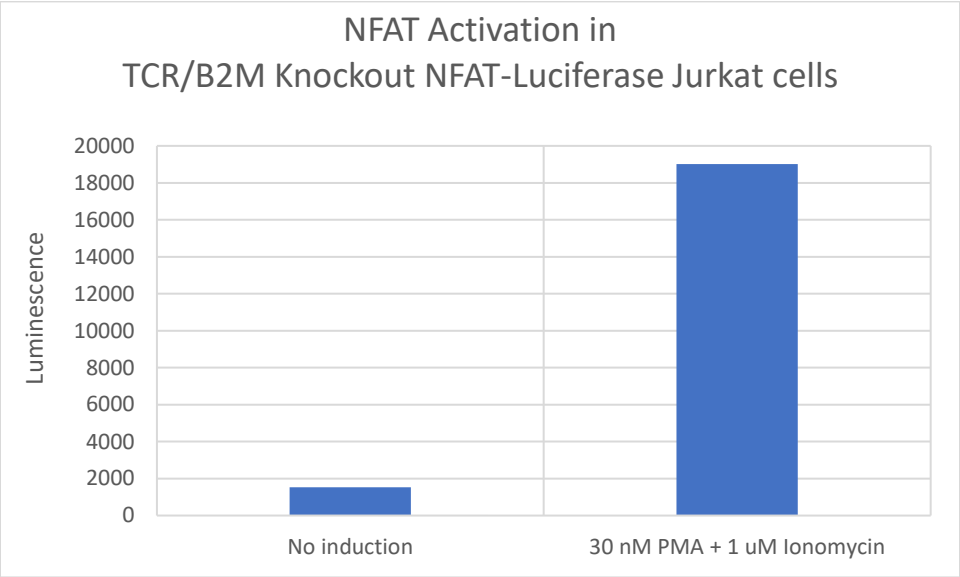

Figure 7: Stimulation of NFAT-Luciferase activity by Ionomycin/PMA.
TCR/B2M Knockout NFAT-Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a white, clear-bottom 96-well plate. Cells were incubated with 1 µM Ionomycin and 30 nM PMA for 24 hours at 37°C. Luciferase activity was measured using the ONE-Step Luciferase reagent (BPS Bioscience #60690).

Sequence
Human beta-2-microglobulin (B2M) mRNA (NCBI Reference Sequence: NM_004048.4), with the sgRNA targeting sequence underlined:

ATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCT
GGAGGCTATCCAGCGTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCCTGAATTGC
TATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTCA
GACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTG
CCGTGTGAACCATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGATCGAGACATGTAAGCAGCATCATGGAGGTTTGA
AGATGCCGCATTTGGATTGGATGAATTCCAAATTCTGCTTGCTTGCTTTTTAATATTGATATGCTTATACACTTACACTTTATG
CACAAAATGTAGGGTTATAATAATGTTAACATGGACATGATCTTCTTTATAATTCTACTTTGAGTGCTGTCTCCATGTTTGAT
GTATCTGAGCAGGTTGCTCCACAGGTAGCTCTAGGAGGGCTGGCAACTTAGAGGTGGGGAGCAGAGAATTCTCTTATCCAA
CATCAACATCTTGGTCAGATTTGAACTCTTCAATCTCTTGCACTCAAAGCTTGTTAAGATAGTTAAGCGTGCATAAGTTAACT
TCCAATTTACATACTCTGCTTAGAATTTGGGGGAAAATTTAGAAATATAATTGACAGGATTATTGGAAATTTGTTATAATGAA
TGAAACATTTTGTCATATAAGATTCATATTTACTTCTTATACATTTGATAAAGTAAGGCATGGTTGTGGTTAATCTGGTTTATT
TTTGTTCCACAAGTTAAATAAATCATAAAACTTGA

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

ATGCTCCTGCTGCTCGTCCCAGTGCTCGAGGTGATTTTTACCCTGGGAGGAACCAGAGCCCAGTCGGTGACCCAGCTTGGC
AGCCACGTCTCTGTCTCTGAAGGAGCCCTGGTTCTGCTGAGGTGCAACTACTCATCGTCTGTTCCACCATATCTCTTCTGGTA
TGTGCAATACCCCAACCAAGGACTCCAGCTTCTCCTGAAGTACACATCAGCGGCCACCCTGGTTAAAGGCATCAACGGTTTT
GAGGCTGAATTTAAGAAGAGTGAAACCTCCTTCCACCTGACGAAACCCTCAGCCCATATGAGCGACGCGGCTGAGTACTTC

TGTGCTGTGAGTGATCTCGAACCGAACAGCAGTGCTTCCAAGATAATCTTTGGATCAGGGACCAGACTCAGCATCCGGCCA
AATATCCAGAACCCTGACCCTGCCGTGTACCAGCTGAGAGACTCTAAATCCAGTGACAAGTCTGTCTGCCTATTCACCGATTT
TGATTCTCAAACAAATGTGTCACAAAGTAAGGATTCTGATGTGTATATCACAGACAAAACTGTGCTAGACATGAGGTCTATG
GACTTCAAGAGCAACAGTGCTGTGGCCTGGAGCAACAAATCTGACTTTGCATGTGCAAACGCCTTCAACAACAGCATTATTC
CAGAAGACACCTTCTTCCCCAGCCCAGAAAGTTCCTGTGATGTCAAGCTGGTCGAGAAAAGCTTTGAAACAGATACGAACCT
AAACTTTCAAAACCTGTCAGTGATTGGGTTCCGAATCCTCCTCCTGAAAGTGGCCGGGTTTAATCTGCTCATGACGCTGCGG
CTGTGGTCCAGCTGA

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

ATGGGCTGCAGGCTGCTCTGCTGTGCGGTTCTCTGTCTCCTGGGAGCAGTTCCCATAGACACTGAAGTTACCCAGACACCAA
AACACCTGGTCATGGGAATGACAAATAAGAAGTCTTTGAAATGTGAACAACATATGGGGCACAGGGCTATGTATTGGTACA
AGCAGAAAGCTAAGAAGCCACCGGAGCTCATGTTTGTCTACAGCTATGAGAAACTCTCTATAAATGAAAGTGTGCCAAGTC
GCTTCTCACCTGAATGCCCCAACAGCTCTCTCTTAAACCTTCACCTACACGCCCTGCAGCCAGAAGACTCAGCCCTGTATCTCT
GCGCCAGCAGCCCCCTCGGGAGGGAGGGACTGAACACTGAAGCTTTCTTTGGACAAGGCACCAGACTCACAGTTGTAGAG
GACCTGAACAAGGTGTTCCCACCCGAGGTCGCTGTGTTTGAGCCATCAGAAGCAGAGATCTCCCACACCCAAAAGGCCACA
CTGGTGTGCCTGGCCACAGGCTTCTTCCCCGACCACGTGGAGCTGAGCTGGTGGGTGAATGGGAAGGAGGTGCACAGTGG
GGTCAGCACGGACCCGCAGCCCCTCAAGGAGCAGCCCGCCCTCAATGACTCCAGATACTGCCTGAGCAGCCGCCTGAGGGT
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ACCCAGGATAGGGCCAAACCCGTCACCCAGATCGTCAGCGCCGAGGCCTGGGGTAGAGCAGACTGTGGCTTTACCTCGGT
GTCCTACCAGCAAGGGGTCCTGTCTGCCACCATCCTCTATGAGATCCTGCTAGGGAAGGCCACCCTGTATGCTGTGCTGGTC
AGCGCCCTTGTGTTGATGGCCATGGTCAAGAGAAAGGATTTCTGA

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.

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*
B2M (Human) CRISPR/Cas9 Lentivirus (Integrating)	78340	500 µl x2
B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78341	500 µl x2
NFAT Reporter (Luc) – Jurkat Recombinant Cell Line	60621	2 vials
TCR Knockout NFAT-Luciferase Reporter Jurkat Recombinant Cell Line	79887	2 vials
B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78363	2 vials