Description

TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line is a Jurkat cell line where TCR (T Cell Receptor) was knockout. The TRAC (T-Cell Receptor Alpha Constant) and TRBC1 (T-Cell Receptor Beta Constant 1) domains of the TCRα/β chains were genetically removed by CRISPR/Cas9 genome editing from Jurkat cells stably expressing the firefly luciferase reporter under the control of NFAT response elements.

This cell line has been functionally validated and does not respond to anti-CD3 agonist antibodies, as opposed to the parental NFAT-Luciferase Reporter Jurkat cells (BPS Bioscience #60621).

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) in T cells. NFAT is regulated by Ca2+ and the Ca2+/calmodulin-dependent serine phosphatase, calcineurin.

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 used 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 loss of TCR expression.

Application(s)

Use as a control to determine CAR-T-specific cell killing.

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
Growth Medium 2B	BPS Bioscience #79530

Materials Used in the Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
NFAT-Luciferase Reporter Jurkat cells	BPS Bioscience #60621
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
Luminometer	
96-well tissue culture plate, white, clear bottom	

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. However, Growth Media *do* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene(s) of interest.

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, and 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 Used in Functional Cellular Assay

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

Cell Culture Protocol

Note: Jurkat cells are derived from human material and thus the use of adequate safety precautions is recommended.

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 Growth Medium 2B.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium 2B. 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

Loss of expression of TCRα/β in the TCR Knockout NFAT-Luciferase Reporter Jurkat cells was confirmed by genomic sequencing and by flow cytometry.

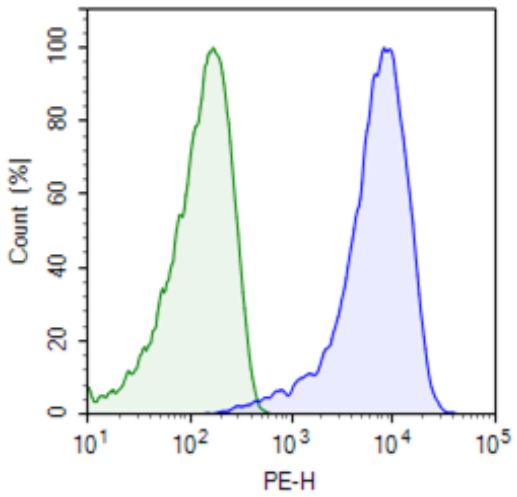

Figure 1: Analysis of TCRα/β expression in TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line.
TCR Knockout NFAT-Luciferase Reporter Jurkat cells were incubated with a PE anti-human α/β T Cell Receptor Antibody (BioLegend #306707) and analyzed by flow cytometry. Parental NFAT-Luciferase Jurkat cells are shown in blue, compared to the TCR Knockout NFAT-Luciferase Reporter Jurkat cells (green). Y-axis represents the % cell number. X-axis indicates PE intensity.

Sequences

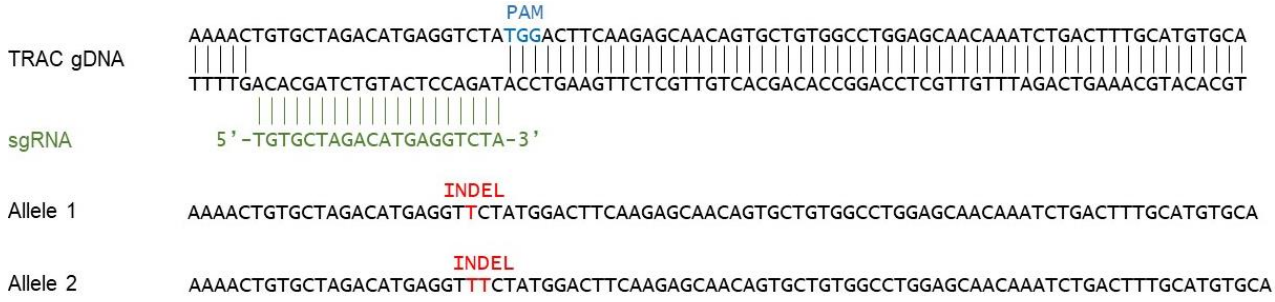

Figure 2. Genomic sequencing of TRAC in the TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line.
The genomic DNA from the TCR Knockout NFAT-Luciferase Reporter Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green,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

Figure 3. Genomic sequencing of TRBC1 in the TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line.
The genomic DNA from the TCR 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.

Functional assay: stimulation of NFAT-Luciferase activity using an anti-CD3 Agonist Antibody.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include "Untreated Cells", "Cell-Free Control" and "Test" conditions.

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1. Centrifuge a suspension culture of TCR Knockout NFAT-Luciferase Reporter Jurkat cells and control NFAT-Luciferase Reporter Jurkat cells and resuspend in Thaw Medium 2 at a density of 5 x 10^5 cells/ml. Leave empty wells as "Cell-Free Control" wells.

2. Prepare a serial dilution of anti-CD3 antibody (from 0 to 10 µg/ml) in Thaw Medium 2 at concentrations 2-fold higher than the desired final concentrations (50 µl/well).

3. For each "Test" well, mix 50 µl of anti-CD3 agonist antibody with 50 µl of the TCR Knockout or control NFAT-Luciferase Reporter Jurkat cells and plate 100 µl of the mix in a white clear-bottom 96-well plate.

   Note: The final cell density is 25,000 cells/100 µl/well.

4. Add 50 µl of Thaw Medium 2 to 50 µl of cells to the "Untreated Control" wells.

5. Add 100 µl of Thaw Medium 2 to "Cell-Free Control" wells (for determining background luminescence).

6. Incubate the plate at 37°C in a CO_2 incubator for 5-6 hours.

7. Add 100 µl of ONE-Step™ Luciferase Reagent to each well.

8. Rock gently at Room Temperature (RT) for ~15 minutes.

9. Measure the luminescence using a luminometer.

10. 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.

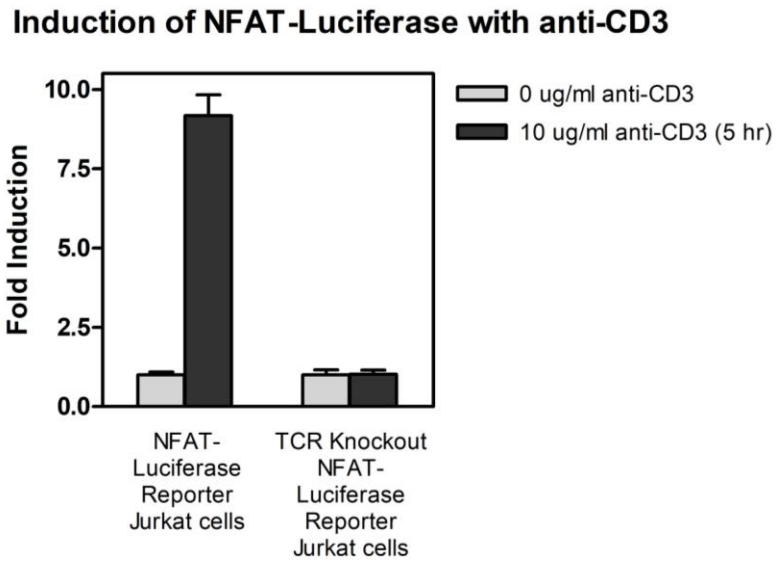

Figure 4: Stimulation of NFAT-Luciferase activity in the TCR Knockout NFAT-Luciferase Jurkat Cell Line by anti-CD3 Agonist Antibody.
Parental NFAT-Luciferase Jurkat cells and TCR Knockout NFAT-Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a white, clear-bottom 96-well plates. Cells were incubated with anti-CD3 agonist antibody at 10 µg/ml for 5 hours at 37°C. Luciferase activity was measured using the ONE-Step™ Luciferase reagent. The anti-CD3 agonist antibody induced luciferase in parental NFAT-Luciferase Reporter Jurkat cells, which express a functional TCR, but not in the TCR Knockout NFAT-Luciferase Jurkat cells, confirming the functional knockout of TCR.

Functional assay: stimulation of NFAT-Luciferase activity using Ionomycin/PMA.

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- The assay should include “Untreated Cells”, “Cell-Free Control” and “Test” conditions.

1. Seed TCR Knockout NFAT-Luciferase Jurkat cells at a density of 25,000 cells per well in 50 µl of Thaw Medium 2 into white clear-bottom 96-well plate. Leave empty wells as “Cell-Free Control” wells.

2. Prepare a solution of Ionomycin and PMA by diluting it into Thaw Medium 2 at a concentration 2-fold higher than the desired final concentration (50 µl/ well).

3. Add 50 µl of the diluted compound to each “Test” well (the final volume is 100 µl).

4. Add 50 µl of Thaw Medium 2 to the “Untreated Control” wells.

5. Add 100 µl of Thaw Medium 2 to “Cell-Free Control” wells (for determining the background luminescence).

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

7. Add 100 µl of ONE-Step™ Luciferase Reagent to each well.

8. Rock gently at RT for ~15 minutes.

9. Measure the luminescence using a luminometer.

10. 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.

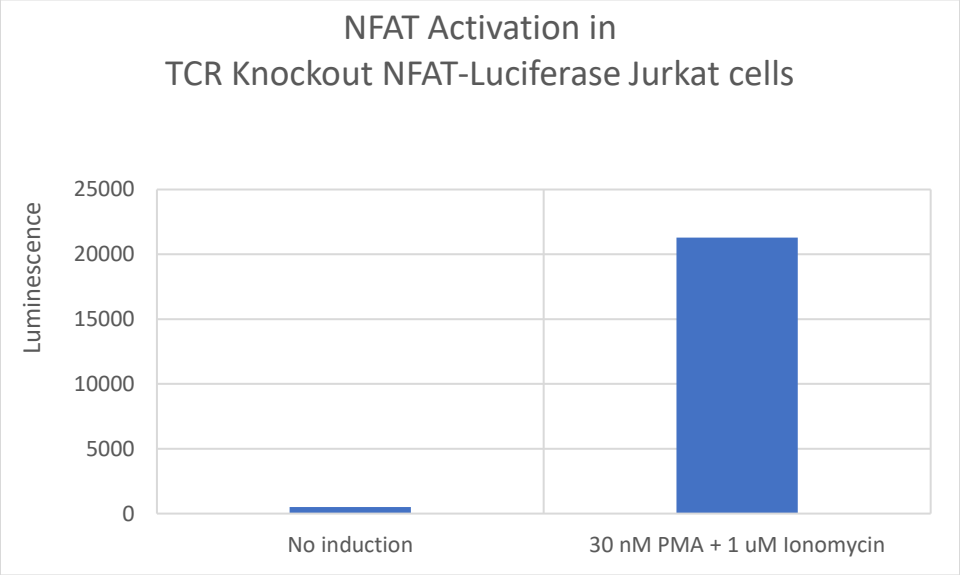

Figure 5: Stimulation of NFAT-Luciferase activity in the TCR Knockout NFAT-Luciferase Jurkat Cell Line by Ionomycin/PMA.
TCR Knockout NFAT-Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a white, clear-bottom 96-well plate. Cells were incubated with 1 µM Ionomycin and 30 nM PMA for 24 hours at 37°C. Luciferase activity was measured using the ONE-Step™ Luciferase reagent.

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

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

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

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*
NFAT Reporter (Luc) Jurkat Recombinant Cell Line	60621	2 vials
Anti-CD28 Agonist Antibody	100182	various
Anti-CD28 Agonist Antibody (Humanized)	100186	various
PD-1 / NFAT Reporter - Jurkat Recombinant Cell Line	60535	2 vials
TCR CRISPR/Cas9 Lentivirus (Non-Integrating)	78062	500 µl x 2
TCR Activator CHO Recombinant Cell line	60539	2 vials

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