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Produktinformation

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

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

This cell line is a knockout of TCR (T Cell Receptor). 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 recombinant Jurkat cells stably expressing the firefly luciferase gene 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 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 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 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, 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°C 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, 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

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 cells/ml. At first passage and subsequent passages, use Growth Medium 2B **(contains Geneticin)**.

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 **(contains Geneticin)**. 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 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

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

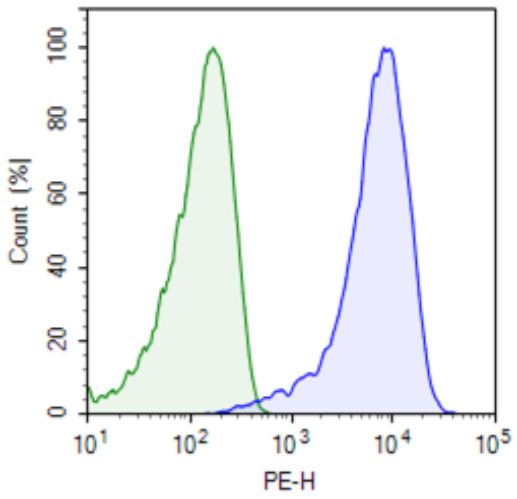

Figure 1: Analysis of TCRα/β Expression in TCR Knockout NFAT-Luciferase Reporter Jurkat cells. TCR Knockout NFAT-Luciferase Reporter Jurkat cells were incubated with a PE-labeled anti-human TCRα/β antibody (BioLegend #306707) and analyzed by flow cytometry. Parental NFAT-Luciferase Jurkat cells are shown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

Sequences

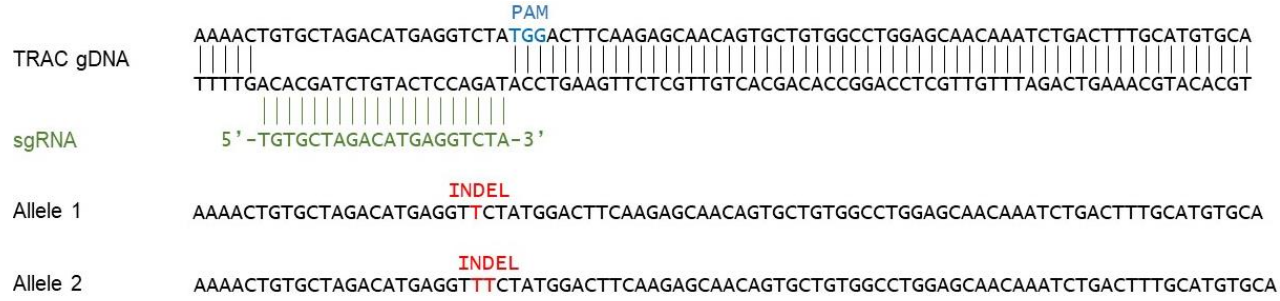

Figure 2. Genomic Sequencing of TRAC in the TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line.
The genomic DNA from the TCR Knockout NFAT-Luciferase Reporter Jurkat cells was isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) in green, 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 cells.
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 assay should be performed in triplicates.

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.

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

3. For each 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 for each well of the "Untreated control".

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

8. 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.

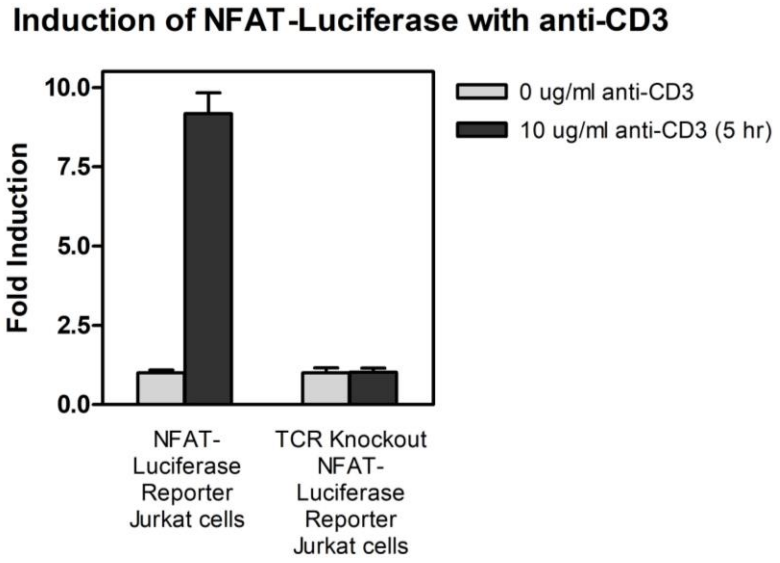

Figure 4: Stimulation of NFAT-Luciferase activity by anti-CD3 Agonist Antibody.
Parental NFAT-Luciferase Jurkat cells and TCR Knockout NFAT-Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a white, clear-bottom 96-well plte. Cells were incubated with anti-CD3 agonist antibody at 10 µg/ml (BPS Bioscience #71274) for 5 hours at 37°C. Luciferase activity was measured using the ONE-Step Luciferase reagent (BPS Bioscience #60690). 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 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 plate 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 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 cells (unstimulated control)

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.

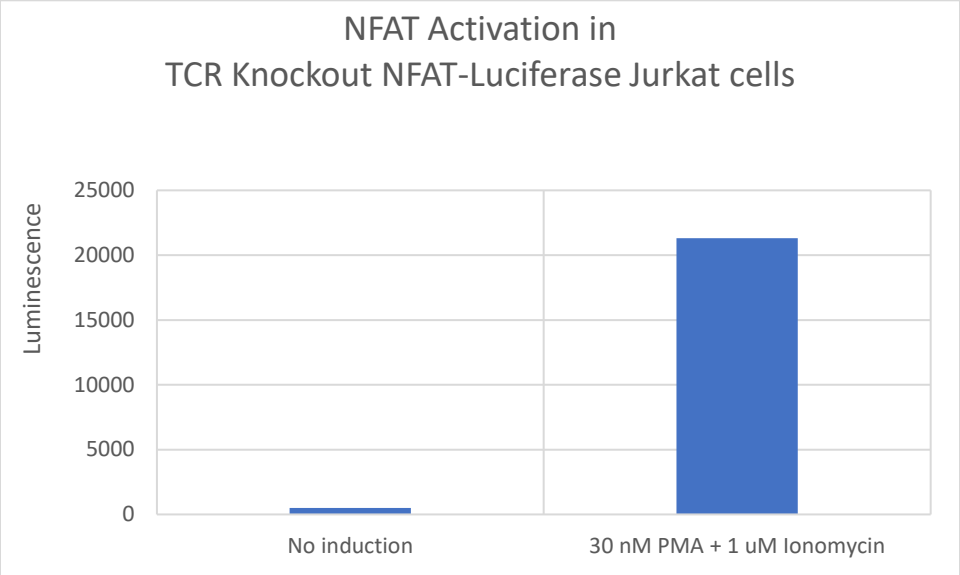

Figure 5: Stimulation of NFAT-Luciferase activity by Ionomycin/PMA.
TCR Knockout NFAT-Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a white, clear-bottom 96-well plate. Cells were incubated with 1 µM Ionomycin and 30 nM PMA for 24 hours at 37°C. Luciferase activity was measured using the ONE-Step Luciferase reagent (BPS Bioscience #60690).

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

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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*
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