Description

This cell line was generated by transducing the TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line (BPS Bioscience #78556) with lentiviruses (CD4 Lentivirus, Bioscience #78987) to overexpress human CD4 (NM_000616.5). To achieve 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 Jurkat cells that stably expressed firefly luciferase under the control of NFAT response elements.

TCRα/β knockout in the TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line was confirmed by both genomic sequencing and flow cytometry. Expression of CD4 in CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line was confirmed by flow cytometry. The cell line does not respond to anti-CD3 agonist antibodies, as opposed to the parental NFAT-Luciferase Reporter Jurkat Cell Line (BPS Bioscience #60621). This cell line has been functionally validated by activation of a transgenic Influenza A Virus HA TCR by the cognate Influenza HA H3 Peptide and MHCII-expressing Antigen Presenting Cells.

Background

The TCR (T Cell Receptor) protein complex 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 Ca^{2+} and the Ca^{2+}/calmodulin-dependent serine phosphatase calcineurin.

CD4 is a cell surface glycoprotein found on partially defined functional T cell subsets, including helper T cells and T-regulatory cells, peripheral monocytes and other APCs (antigen-presenting cells). The ectodomain of CD4 binds to membrane-proximal domains of MHC class II molecules, while its cytoplasmic domain interacts with the protein tyrosine kinase p56lck (lck) through a shared cysteine-containing motif. CD4+ T cells, or helper T cells, are one type of lymphocyte that helps coordinate the immune response against infection and disease by activating cells of the innate immune system, B lymphocytes, and cytotoxic T cells. CD4+ T cells are activated by interaction between the TCR (T cell receptor) and its cognate peptide presented on MHC II molecules, and CD4 is a critical component of the T cell receptor complex that recognizes antigenic peptides presented by MHC II molecules, increasing its stability.

Application

- Study transgenic TCR expression by introducing TCR alpha and beta chains.
- Study the activation of introduced TCRs by cognate peptide(s) and MHCII-expressing APCs (Antigen Presenting 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.

Name	Ordering Information
Cells of interest	
A375 Cells	ATCC #CRL-1619
TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78556
Growth Medium 2C	BPS Bioscience #79592
Assay Medium 2D	BPS Bioscience #78755
Thaw Medium 6	BPS Bioscience #60183
Thaw Medium 2	BPS Bioscience #60184
PE anti-human α/β T Cell Receptor Antibody	BioLegend #306707
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
Influenza A Virus HA TCR Lentivirus (Clone HA1.7)	BPS Bioscience #78988
Influenza HA H3 Peptide (307-319)	BPS Bioscience #82311
Influenza HA H1 Peptide (Hawaii H1N1)	BPS Bioscience #82312
Lenti-Fuse™ Polybrene Viral Transduction Enhancer	BPS Bioscience #78939
96-well tissue culture-treated assay plates	
Flow cytometer	

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 BPS Bioscience validated and optimized media *is highly recommended*. Other preparations or formulations of media may result in suboptimal performance.

Media Required for Maintaining CD4+ TCR Knockout NFAT-Luciferase Reporter Cell Line

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, and 1% Penicillin/Streptomycin.

Growth Medium 2C (BPS Bioscience #79592):

RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin, 100 µg/ml Hygromycin B.

Media Required for Maintaining A375 Cell Line

Thaw Medium 6 (BPS Bioscience #60183):

DMEM medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Media Required for Co-culture Assay

Assay Medium 2D (BPS Bioscience #78755):
RPMI 1640 medium (ATCC modification) supplemented with 1% FBS.

Cell Culture Protocol

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

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**.**

   Note: 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.

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, 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 2C.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, with Growth Medium 2C. 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 storage.

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

Functional Assay Protocol

- The following protocol was used to transduce a Jurkat cell line. The transduction conditions (e.g. MOI, concentration of polybrene, time of assay development) should be optimized according to the cell type and the assay requirements. In most cell types, the expression of the reporter gene can be measured approximately 48-72 hours after transduction. For cell types with low transduction efficacy, it may be necessary to select the cells stably expressing the reporter with puromycin prior to carrying out the reporter assays.
- The assay should include "Peptide Loaded" and "Unloaded Control" wells.
- We recommend using TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line (#78556) as negative control.

Day 1:

1. Harvest CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2C by centrifugation, resuspend the cells in fresh Thaw Medium 2 and count.

2. Dilute cells to a density of 2 x 10^5 cells/ml in Thaw Medium 2.

3. Mix 1 ml of the Jurkat cell suspension with the appropriate amount of Influenza A Virus HA TCR Lentivirus (Clone HA1.7) in a 1.5-ml Eppendorf tube to obtain an MOI > 10.

4. Add Lenti-Fuse™ Polybrene Viral Transduction Enhancer to a final concentration of 8 µg/ml.

5. Gently mix and incubate the virus with the Jurkat cells for 20 minutes at Room Temperature (RT) in a tissue culture hood.

6. Centrifuge the virus/cell mixture for two hours at 800 *x g* and 32°C (spinoculation).

7. Add the cells/virus mix from the spinoculation step to one well of a 6-well plate.

8. Add an additional 1.5 ml of Thaw Medium 2 to the well.

   Note: It is not necessary to remove the virus.

9. Incubate the cells at 37°C with 5% CO_2 for 48-66 hours.

Day 3-4:

1. The expression of TCR can be analyzed by flow cytometry. The transduced Jurkat cells are ready for assay development on day 3 or 4.

   Note: If the transduction efficiency is low, it may be necessary to perform cell selection with puromycin on day 3.

2. Plate A375 cells as APCs (Antigen Presenting Cells) at a density of 2 x 10^4 cells/well to test the TCR mediated reporter activation.

3. Incubate overnight at 37°C with 5% CO_2.

Day 3-4:

1. For use in the following co-culture assay at day 4-5 prepare materials and conditions as follows:

   1.1 Preparation of Antigenic-Mimetic Peptides:

   a) Thaw the peptide at RT.

   b) Dilute the peptide with Assay Medium 2D so that it is 2-fold higher than the desired final concentration (50 µl/well).

   Note: The peptide stock was dissolved in DMSO at a concentration of 1 mM. The final DMSO concentration in the co-culture assay should not be >0.3%.

   1.2 Preparation of Antigen Presenting Cells (APCs):

   a) Remove the media from plated A375 cells.

   b) Add 50 µl of diluted peptide to the "Peptide Loaded" wells.

   c) Add 50 µl of Assay Medium 2D to the "Unloaded Control" wells (for measuring the basal luciferase activity).

2. Resuspend TCR-transduced CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells in Assay Medium 2D at a density of 5 x 10^5 cells/ml (50 µl/well).

3. Add 50 µl of TCR-transduced CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the APCs.

4. Incubate the co-culture plate at 37°C with 5% CO_2 for 5-6 hours or overnight.

Day 5-6:

1. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well.

2. Incubate at RT for ~15 to 30 minutes.

3. Measure luminescence using a luminometer.

Validation Data

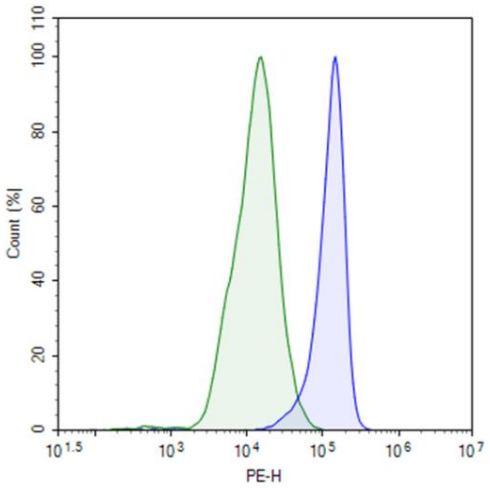

Figure 1. Analysis of CD4 expression in CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line by flow cytometry.
Approximately 10,000 of CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (blue) and TCR Knockout NFAT- Luciferase Reporter Jurkat cells (BPS Bioscience #78556) (green) were stained with Anti-CD4 Antibody, PE-Labeled (BPS Bioscience #102010) and analyzed by flow cytometry. The y-axis represents the cell % and the x-axis indicates PE intensity.

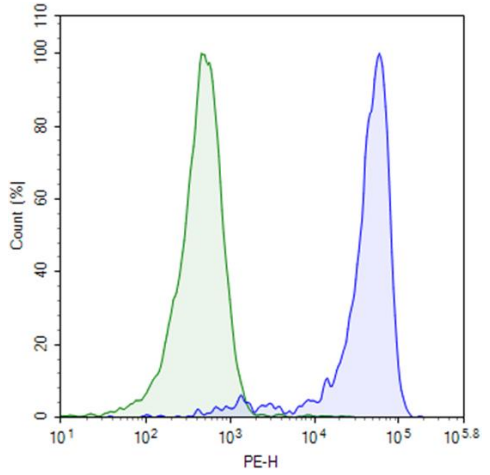

Figure 2: Expression of Influenza A HA TCR in CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line transduced with TCR lentiviruses.
Approximately 100,000 CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells were transduced with Influenza A Virus HA TCR Lentivirus (Clone HA1.7) (BPS Bioscience #78988) by spinoculation at a MOI of 10. 48 hours post-transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, the cell pool was stained with PE anti-human α/β T Cell Receptor Antibody (BioLegend #306707) and the expression of Influenza A HA TCR was analyzed by flow cytometry (blue). Non-transduced cells were used as control (green). The y axis represents the % of cells. The x axis indicates fluorophore intensity.

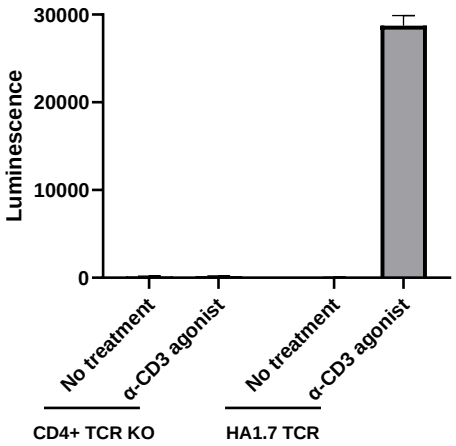

Figure 3: Expression of Influenza A HA TCR in CD4+ TCR Knockout NFAT-Luciferase Reporter Cell Line confers responsiveness to an anti-CD3 agonist, which induces NFAT-dependent luciferase activity.

CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells were transduced with Influenza A Virus HA TCR Lentivirus (Clone HA1.7) (BPS Bioscience #78988) via spinoculation at a MOI of 10. 48 hours post-transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, 20,000 of transduced (HA1.7 TCR) or control (CD4+ TCR KO) cells transferred to a 96-well plate pre-coated with 1 µg/ml of Anti-CD3 Agonist Antibody (BPS Bioscience #71274) for 6 hours. Non-coated wells were run in parallel as controls. Results are shown as raw luminescence readings.

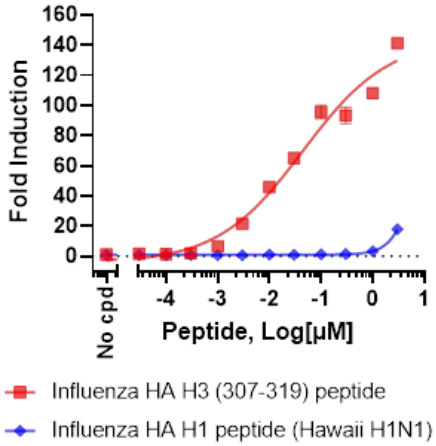

Figure 4: CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat T Cell Line activation after transduction with Influenza A Virus HA TCR Lentivirus (Clone HA1.7), using A375 cells as Antigen presenting cells (APCs).

CD4+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells were transduced with Influenza A Virus HA TCR Lentivirus (Clone HA1.7) (BPS Bioscience #78988) by spinoculation at a MOI of 10. 48 hours post-transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, the cell pool was co-cultured with A375 cells (ATCC #CRL-1619) loaded with Influenza HA H3 Peptide (307-319) (BPS Bioscience #82311) or control Influenza HA H1 Peptide (Hawaii H1N1) (BPS Bioscience #82312) for 6 hours. Luciferase activity was measured with ONE-Step™ Luciferase Assay System and correlates with T Cell Activation.

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.

References

Janeway C.A. Jr., 1991 *Semin Immunol.* 3(3):153-60.
Fleury S.G., *et al.,* 1991 *Semin Immunol.* 3(3):177-85.
Hennecke J. and Wiley D.C., 2002 *J Exp Med.* 195:571–581.

Related Products

Products	*Catalog #*	*Size*
CD8a Lentivirus	78648	500 µl x 2
CD8a/CD8b Lentivirus	78650	500 µl x 2
Human CD4 Lentivirus	78987	500 µl x 2
Anti-CD4, PE-Labeled	102010	25 µg/100 µg
Anti-CD8, PE-Labeled	102011	25 µg/100 µg
CD8+ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	78757	2 vials

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