Description

This cell line was generated from TCR Knockout NFAT Luciferase Reporter Jurkat Cell Line (BPS Bioscience #78556) by exogeneous overexpression of human CD8 (NM_001768.6) using lentiviral transduction (CD8a Lentivirus, BPS Bioscience #78648). 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 recombinant Jurkat cells stably expressing the firefly luciferase gene under the control of NFAT response elements.

TCRα/β knockout in the TCR Knockout NFAT-Luciferase Reporter Jurkat cells was confirmed by genomic sequencing and by flow cytometry and expression of CD8 was confirmed by flow cytometry. The 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) 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.

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)

- Study transgenic TCR expression by introducing TCR alpha and beta chains.
- Study the activation of transgenic TCR by cognate peptide(s) and MHC1-expressing APCs (Antigen Presenting 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.

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2C	BPS Bioscience #79592
Assay Medium 2D	BPS Bioscience #78755
TCR KO NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78556
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
NY-ESO-1 Specific TCR Lentivirus (1G4)	BPS Bioscience #78675
MART-1 Specific TCR Lentivirus (DMF4)	BPS Bioscience #78678
T2 Cell Line	ATCC #CRL-1992
NY-ESO-1 (157-165) Peptide	BPS Bioscience #78758
MART-1 (Leu26-35, Leu27) Peptide	BPS Bioscience #78760
APC MHC I Dextramer (HLA-A*02:01 SLLMWITQV)	Immudex #WB03247
APC MHC I Dextramer (HLA-A*02:01 ELAGIGILTV)	Immudex #WB02162
Alexa Fluor 647™ Anti-human CD8	Biolegend #344725
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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 by 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 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 (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 of Geneticin and 100 µg/ml Hygromycin B.

Media Used in Functional Cellular Assay

Assay Medium 2D (BPS Bioscience #78755):

RPMI 1640 medium (ATCC modification) supplemented with 1% FBS

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 or Hygromycin)**.
   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 or Hygromycin)**.

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 or Hygromycin)**, 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 **(contains Geneticin and Hygromycin)**.

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 2C **(contains Geneticin and Hygromycin)**. 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 $CD8^+$ TCR Knockout NFAT Luciferase Reporter Jurkat cell line with a BPS Bioscience TCR lentivirus. The anti-CD3 agonist response and NFAT-dependent Luciferase activity of the TCR-transduced cells was evaluated.

1. Day 1: Harvest the $CD8^+$ TCR knockout NFAT Luciferase Reporter Jurkat cells from Growth Medium 2C by centrifugation and resuspend the cells in fresh Thaw Medium 2. Dilute the cells to a density of 2 x 10^5/ml in Thaw Medium 2. Mix 1 ml of the Jurkat cells with NY-ESO-1-specific or MART-1-specific TCR Lentivirus in a 1.5-ml Eppendorf tube at an MOI>10.

   Add polybrene to a final concentration of 8 µg/ml. Gently mix and incubate the virus with the Jurkat cells for 20 minutes at room temperature in a tissue culture hood.

2. Centrifuge the virus/cell mix for two hours at 800 *x g* at 32°C (spinoculation). Add the virus/cell mix from the spinoculation step to one well of a 6-well plate. Add an additional 1.5 ml of Thaw Medium 2 to the well. It is not necessary to remove the virus. Incubate the cells at 37°C with 5% CO_2 for 48-66 hours.

   The expression of TCR can be analyzed by Flow cytometry. The transduced Jurkat cells are ready for assay development on day 3 or 4. If the transduction efficiency is low, it may be necessary to perform cell selection with puromycin on day 3.

3. For use in the following co-culture assay, Day 4 prepare materials as follows:

   a) Preparation of Antigenic Peptides:

      Thaw the NY-ESO-1 or MART-1 peptide at room temperature. Dilute the peptide with Assay Medium 2D so that it is 5-fold higher than the desired final concentration.

      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%.

   b) Preparation of Antigen Presenting Cells (APCs):
      Harvest T2 cells (Antigen Presenting Cells, APC) from Thaw Medium 2 and resuspend the cells into Assay Medium 2D at a density of 5 x 10^5/ml. Add 40 µl of T2 cells into each well of a 96-well plate.

      Add 20 µl of diluted peptide to the “peptide stimulated” APC wells. Add 20 µl of Assay Medium 2D to the “unstimulated control” APC wells (for measuring the basal luciferase activity).

   c) Resuspend Jurkat cells into Assay Medium 2D at a density of 4 x 10^5/ml. Add 40 µl of TCR-transduced $CD8^+$ TCR knockout NFAT Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the antigen-loaded and control APCs.

4. Incubate the co-culture plate at 37°C with 5% CO2 for 5-6 hours or overnight.

5. Prepare the ONE-Step™ Luciferase reagent per recommended protocol. Add 100 µl of ONE-Step™ Luciferase Assay reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

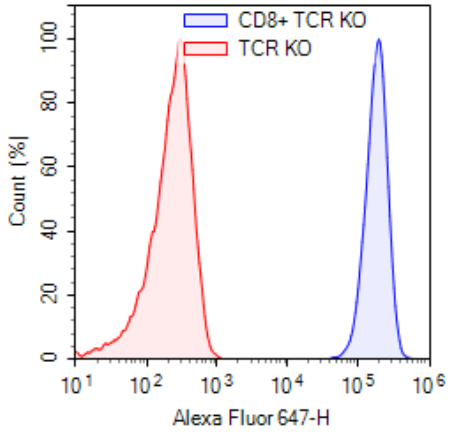

Figure 1: Expression of CD8 in CD8⁺ TCR Knockout NFAT-Luciferase Reporter Jurkat cells.
Approximately 100,000 CD8⁺ TCR Knockout NFAT-Luciferase Reporter Jurkat cells were incubated with an Alexa Fluor® 647 anti-human CD8 Antibody (Biolegend #344725) and analyzed by flow cytometry. Parental TCR knockout NFAT-Luciferase Jurkat cells are shown in red, compared to the CD8⁺ TCR Knockout NFAT-Luciferase Reporter Jurkat cells shown in blue.

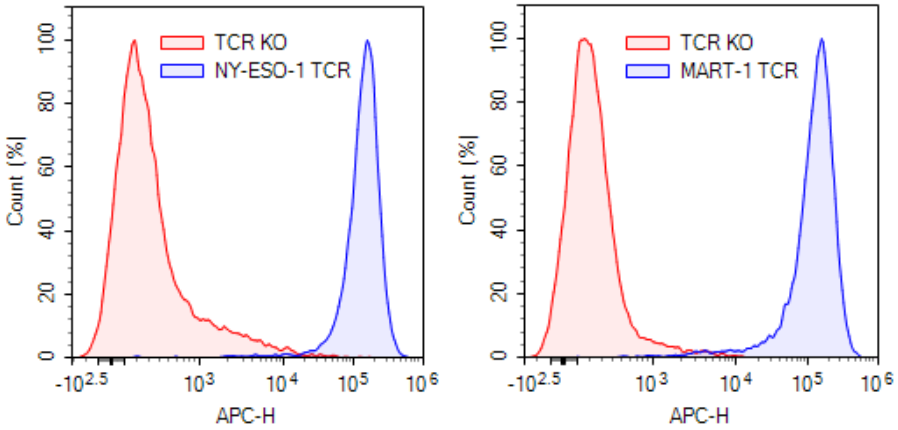

Figure 2: Expression of transgenic TCR in Jurkat cells transduced with TCR lentiviruses.
Approximately 100,000 CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were transduced with NY-ESO-1-specific TCR Lentivirus (1G4, BPS Bioscience #78675) or MART-1-specific TCR lentivirus (DMF4, BPS Bioscience #78678) via spinoculation at a MOI of 10. After 48 hours of transduction, the cells were transferred into a medium containing 0.5 µg/ml of puromycin. After one week of antibiotic selection, the expression of TCR was analyzed by flow cytometry.
Left: NY-ESO-1-specific TCR lentivirus transduced CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were stained with APC-conjugated MHC-I Dextramer (HLA-A*02:01 SLLMWITQV; Immudex#WB03247), shown in blue. Untransduced CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells are shown in Red.
Right: MART-1-specific TCR lentivirus transduced CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were stained with APC-conjugated MHC-I Dextramer (HLA-A*02:01 ELAGIGILTV; Immudex#WB02162), shown in blue. Untransduced CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells are shown in Red.

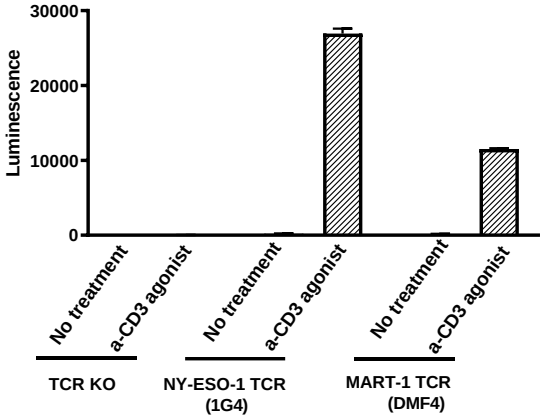

Figure 3: The Expression of Transgenic TCR in CD8⁺ TCR knockout NFAT Luciferase Reporter Cell Line confers responsiveness to anti-CD3 agonist, which induces NFAT-dependent luciferase activity.
Approximately 20,000 CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells (BPS Bioscience #78757)/well (96-well plate) were transduced with NY-ESO-1-specific TCR lentivirus (clone 1G4) (BPS Bioscience #78675) or MART-1-specific TCR (clone DMF4) (BPS Bioscience #78678) via spinoculation at a MOI of 10. After 66 hours of transduction, the medium was changed to Thaw Medium 2. Cells were stimulated by transferring them to a 96-well plate pre-coated with anti-CD3 agonist antibody (BPS Bioscience #71274) at 1 µg/ml for 6 hours. The non-coated wells and the non-transduced cells were run in parallel as controls. Results are shown as raw luminescence readings.

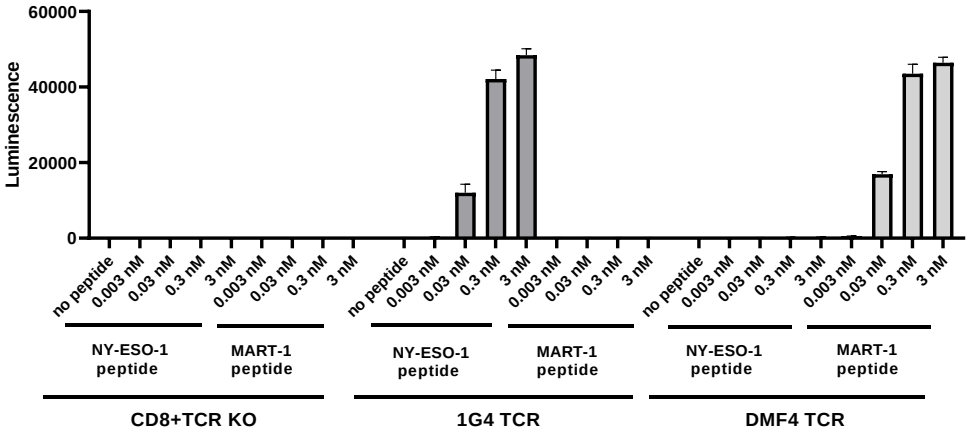

Figure 4: T Cell Activation using T2 cells as APC.
CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were transduced with lentiviruses expressing various TCRs via spinoculation at a MOI of 10. After 66 hours of transduction, the cells were co-cultured with T2 cells loaded NY-ESO-1 peptide (BPS Bioscience #78758) or with MART-1 peptide (BPS Bioscience #78760) for 6 hours. The luciferase assay was performed, and the results are shown as raw luminescence readings. Cells transduced with NY-ESO-1-specific TCR (clone 1G4) (BPS Bioscience #78675) can be activated by NY-ESO-1 peptide-loaded T2 cells, but not MART-1 peptide-loaded T2 cells, while cells transduced with MART-1-specific TCR (clone DMF4) can be activated by MART-1 peptide-loaded T2 cells, but not NY-ESO-1 peptide-loaded T2 cells. Untransduced CD8⁺ TCR-Knockout NFAT Luciferase reporter cell line, where no TCR is expressed, were run in parallel as a negative control.

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*
NY-ESO-1-Specific TCR Lentivirus (Clone c259)	78676	100 µl/2 x 500 µl
NY-ESO-1-Specific TCR Lentivirus (Clone 1G4)	78675	100 µl/2 x 500 µl
MART-1-Specific TCR Lentivirus (Clone DMF4)	78678	100 µl/2 x 500 µl
MART-1-Specific TCR Lentivirus (Clone DMF5)	78679	100 µl/2 x 500 µl
NY-ESO-1 Peptide (157-165)	78758	100 µl
MART-1 Peptide (26-35)	78759	100 µl
MART-1 Peptide (26-35, Leu 27)	78760	100 µl
MART-1 Peptide (27-35)	78761	100 µl