Description

This cell line was generated from the T Cell Receptor (TCR) Knockout NFAT Luciferase Reporter Jurkat Cell Line (BPS Bioscience #78556) by overexpression of human CD8 (NM_001768.6) and MART-1-specific TCR (DMF4) using lentiviral transduction (CD8a Lentivirus #78648 and MART-1-Specific TCR Lentivirus Clone DMF4 #78678). The human TCR clone DMF4 specifically recognizes tumor antigen MART-1 (Melanoma-associated antigen recognized by T cells-1, or Melan-A).

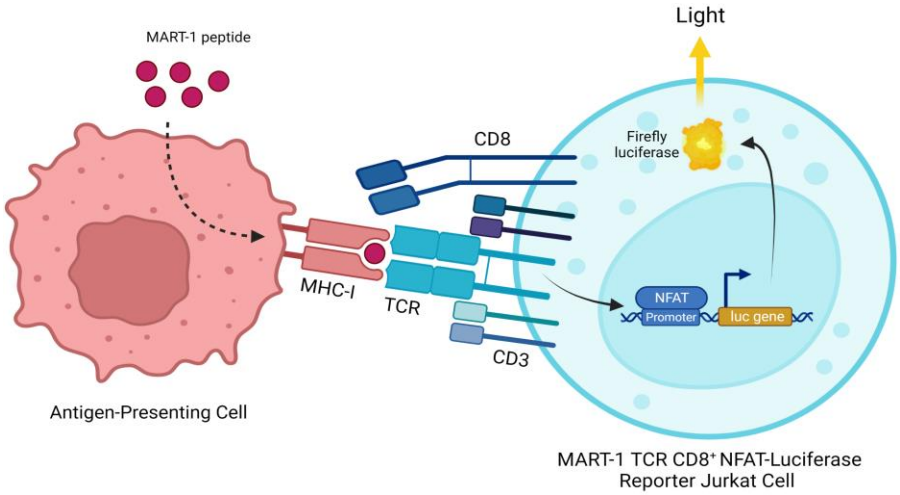

Figure 1: Illustration of the functional co-culture assay used to validate the MART-1 TCR (DMF4) CD8+ NFAT-Luciferase Reporter Jurkat cell line.

Background

MART-1 is a differentiation antigen expressed on the surface of melanocytes. A peptide fragment of the protein is found in MHC complexes that are recognized by CD8+ cytotoxic T cells. MART-1 is present in most skin cancers, including melanomas, and is used as a biomarker for diagnostic purposes. Since the expression of the protein is restricted to melanocyte containing tissues (skin and retina), and is not found in other tissues, MART-1 is an attractive target for cancer vaccines and adoptive cell therapy. The MART-1 peptide 26-35 is a fragment commonly associated with MHC and recognized by T cell receptors.

CD8 (Cluster of Differentiation 8) is a co-receptor of TCR and a typical marker of cytotoxic T cells. The TCR 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) transcription factors that induce the expression of various cytokines such as interleukin-2 to 4, and TNF-alpha. The use of engineered TCR allows T cells to target specific antigens present in cancer cells, via the MHC, expanding the portfolio of antigens that can be targeted in cancer cell therapy.

Application(s)

- Design and optimize co-culture bioassays for MART-1-specific TCR cell evaluation.
- Use as a positive control in experiments evaluating MART-1-specific TCR expressing 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 2T	BPS Bioscience #78756
Assay Medium 2D	BPS Bioscience #78755
$CD8^+$ TCR KO NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78757
T2 Cell Line	ATCC #CRL-1992
MART-1 (26-35) Peptide	BPS Bioscience #78759
MART-1 (26-35, Leu27) peptide	BPS Bioscience #78760
MART-1 (27-35) Peptide	BPS Bioscience #78761
APC MHC I Dextramer (HLA-A*02:01 ELAGIGILTV)	Immudex #WB02162
PE anti-human α/β T Cell Receptor Antibody	Biolegend #306707
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 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 2T (BPS Bioscience #78756):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 mg/ml of Geneticin, 100 µg/ml Hygromycin B, and 0.25 µg/ml puromycin.

Media Used in Functional Cellular Assay

Assay Medium 2D (BPS Bioscience #78755):
RPMI 1640 medium 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.
   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 2T.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 0.2 x 10^6 cells/ml, in Growth Medium 2T. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 - 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.

Functional Co-culture Assay Protocol:

1. Preparation of Antigenic Peptides

   1.1 Thaw the MART-1 peptide at room temperature.

   1.2 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%.

2. Preparation of Antigen Presenting Cells (APCs):

   2.1 Harvest T2 cells (APC) from Thaw Medium 2 and resuspend the cells into Assay Medium 2D at a density of 5 x 10^5/ml.

   2.2 Add 40 µl of T2 cells into each well of a 96-well plate.

   2.3 Add 20 µl of diluted peptide to the "peptide stimulated" APC wells.

   2.4 Add 20 µl of Assay Medium 2D to the "unstimulated control" APC wells (for measuring basal luciferase activity).

3. Harvest the MART-1 TCR (DMF4) $CD8^+$ NFAT Luciferase Reporter Jurkat cells from Growth Medium 2T by centrifugation and resuspend the cells in Assay Medium 2D at a density of 4 x 10^5/ml.

4. Add 40 µl of MART-1 TCR (DMF4) $CD8^+$ NFAT Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the antigen-loaded APCs.

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

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

7. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

Validation Data

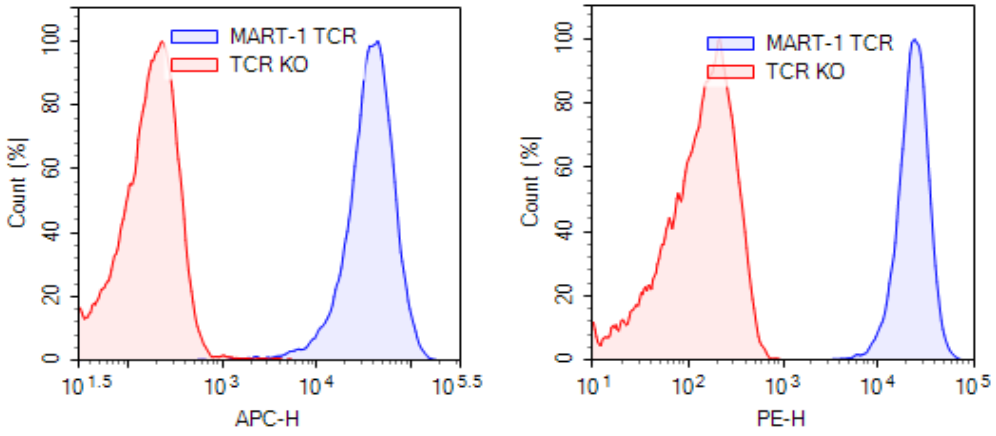

Figure 2: Expression of MART-1 TCR in MART-1 TCR (DMF4) CD8⁺ NFAT-Luciferase Reporter Jurkat cells.
Left: MART-1 TCR (DMF4) CD8⁺ NFAT Luciferase Reporter Jurkat cells were stained with APC-conjugated MHC-I Dextramer (HLA-A*02:01 ELAGIGILTV; Immudex#WB02162) and analyzed by flow cytometry (blue). CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were used as negative control (red).
Right: MART-1 TCR (DMF4) CD8⁺ NFAT Luciferase Reporter Jurkat cells were stained with PE-conjugated anti-human TCR antibody (Biolegend #306707) and analyzed by flow cytometry (blue). CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were used as negative control (red).

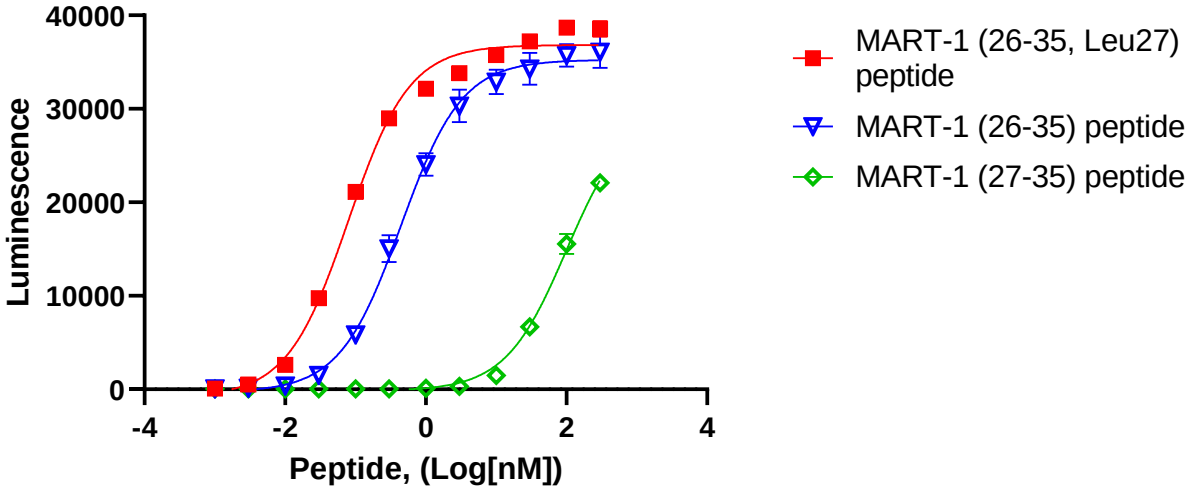

Figure 3: T Cell Activation using T2 cells as APC.
MART-1 TCR (DMF4) CD8⁺ NFAT Luciferase Reporter Jurkat cells were co-cultured for 6 hours with T2 cells loaded with various concentration of MART-1 peptides (BPS Bioscience #78759, #78760 and #78761). MART-1 peptide variants have different affinities for MART-1, with MART-1 (26-35, Leu 27) showing the highest affinity and MART-1 (27-35) peptide the lowest. ONE-Step™ Luciferase Assay was performed, and the results are shown as raw luminescence readings.

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*
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
CD8+ TCR KO NFAT Luciferase Reporter Jurkat Cell Line	78757	2 vials
MART-1 Peptide (26-35)	78759	100 µl
MART-1 Peptide (27-35)	78761	100 µl
MART-1 Peptide (26-35, Leu27)	78760	100 µl