Description

MAGE-A4 CD8+ NFAT-Luciferase Reporter Jurkat Cell Line was generated from T Cell Receptor (TCR) Knockout NFAT Luciferase Reporter Jurkat Cell Line (BPS Bioscience #78556) by overexpression of human CD8 (NM_001768.6) and a MAGE-A4 (Melanoma-associated antigen 4)-directed TCR using lentiviral transduction (CD8a Lentivirus #78648 and MAGE-A4-Specific TCR Lentivirus #78935). This human MAGE-A4 TCR specifically recognizes an antigen corresponding to MAGE-A4 peptide, amino acids 230-239 (GVYDGREHTV).

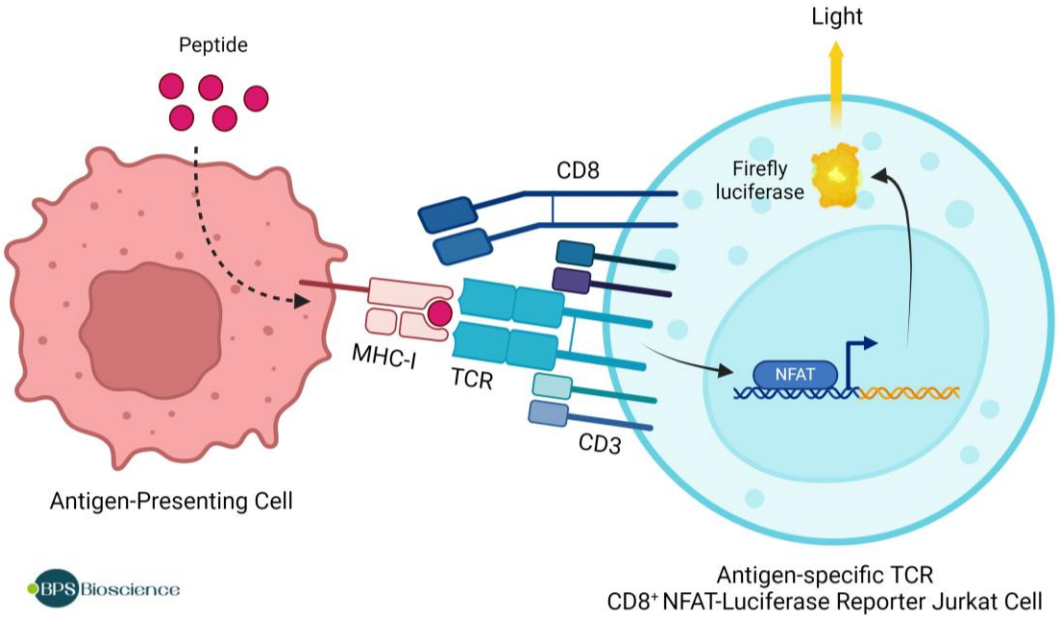

Figure 1: Illustration of the functional co-culture assay used to validate the MAGE-A4 TCR CD8+ NFAT-Luciferase Reporter Jurkat Cell Line.

Background

MAGE (melanoma associated antigen) proteins are CT (cancer testis) antigens, and there are about 60 proteins in the MAGE family that can be subdivided into type I (present only on the X-chromosome, MAGE-A, B, and C) and type II (MAGE D-L and necdin). Under normal conditions they are mostly found in the testis and placenta. They are found at high levels in several cancer types, such as melanoma, brain, and breast cancer, and are involved in the development of resistance to chemotherapy, cell motility, and cell survival. Expression of MAGE proteins tend to correlate with a poor prognosis. They are intracellular proteins, with MAGE-A4 being found in the cytosol and nucleus, making them poor targets for strategies such as CAR-T cell therapy. MAGE proteins are degraded in the proteosome, and the peptides created can then be found on the cell membrane in combination with MHC (major histocompatibility complex) I. Presentation on the cell surface makes them an attractive target for TCR (T cell receptor)-T cell therapy. Several clinical trials are ongoing, and either alone or in combination with other forms of cancer therapy the use of TCR-T cells targeting MAGE-A antigens may become a promising therapeutic avenue.

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.

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Application(s)

- Design and optimize co-culture bioassays for MAGE-A4-specific TCR cell evaluation.
- Use as a positive control in experiments evaluating MAGE-A4 TCR cells.
- Use as an *in vitro* system to measure vaccine T cell immunogenicity.

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
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
MAGE-A4 (230-239) Peptide	BPS Bioscience #78966
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 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.

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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.

   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 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, with 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.

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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 Assay Protocol

- 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 "Peptide Stimulated" and "Unstimulated Control" conditions.
- We recommend using CD8$^+$ TCR KO NFAT Luciferase Reporter Jurkat Cell Line as negative control.

1. Preparation of Antigenic Peptides

   1.1 Thaw the MAGE-A4 Peptide (amino acids 230-239) at Room Temperature (RT).

   1.2 Dilute the peptide with Assay Medium 2D to a concentration 5-fold higher than the desired final concentration (20 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%.

2. Preparation of Antigen Presenting Cells (APCs):

   2.1 Harvest T2 cells (APC) from Thaw Medium 2 and resuspend the cells in 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 MAGE-A4 TCR 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 MAGE-A4 TCR CD8$^+$ NFAT Luciferase Reporter Jurkat cells into each well of the 96-well plate containing the 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 RT for ~15 to 30 minutes and measure luminescence using a luminometer.

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Validation Data

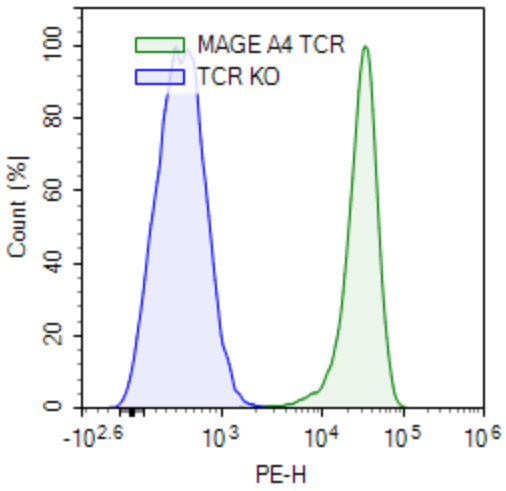

Figure 2: Flow cytometry analysis of the expression of MAGE-A4 TCR in MAGE-A4 TCR CD8⁺ NFAT-Luciferase Reporter Jurkat Cell Line.
MAGE-A4 TCR CD8⁺ NFAT-Luciferase Reporter Jurkat cells (green) and CD8⁺ TCR-Knockout NFAT-Luciferase Reporter Jurkat cells (blue) were stained with PE anti-human α/β T Cell Receptor Antibody (BioLegend #306707) and analyzed by flow cytometry. The y axis represents the % of cells and the x axis the fluorophore intensity.

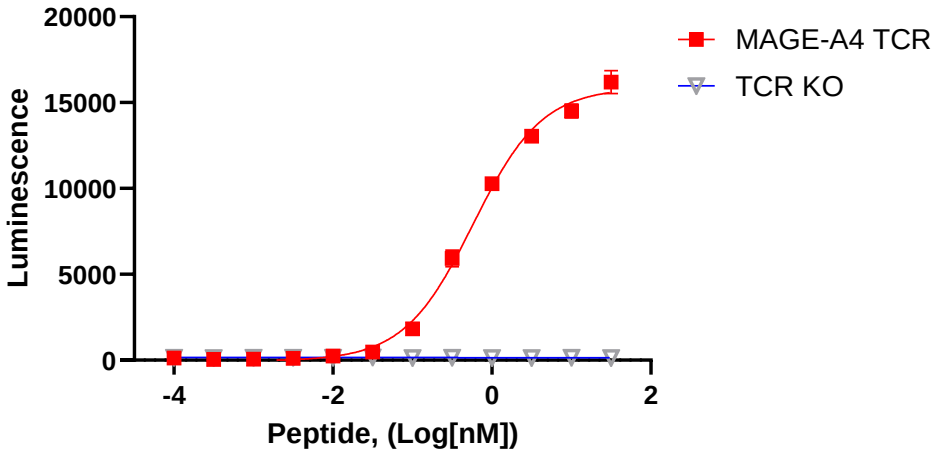

Figure 3: MAGE-A4 TCR CD8⁺ NFAT Luciferase Reporter Jurkat Cell Line activation using T2 cells as APC.
MAGE-A4 TCR CD8⁺ NFAT Luciferase Reporter Jurkat cells were co-cultured overnight with T2 cells loaded with various concentrations of MAGE-A4 Peptide (230-239) (#78966). ONE-Step™ Luciferase assay was performed, and the results are shown as raw luminescence readings. CD8⁺ TCR-Knockout NFAT Luciferase Reporter cells (#78757), where no TCR is expressed, was run in parallel as a negative control.

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

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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*
MAGE-A1 Peptide (278-286)	78965	100 µl
MAGE-A4 Peptide (286-294)	82305	100 µl
MAGE-A1 TCR Lentivirus	78934	100 µl
MAGE-A1 TCR CD8+ NAFT-Luciferase Reporter Jurkat Cell Line	78993	2 Vials

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