

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 NY-ESO-1 TCR (c259) using lentiviral transduction (CD8a Lentivirus #78648 and NY-ESO-1 -Specific TCR Lentivirus Clone c259 #78676). The human TCR clone c259 that specifically recognizes antigen NY-ESO-1 (New York esophageal squamous cell carcinoma 1).

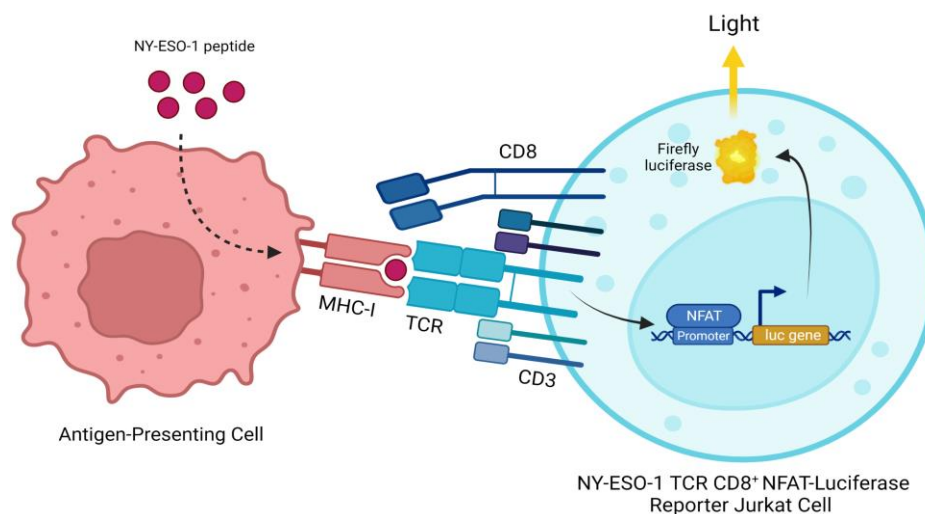


Figure 1: Illustration of the functional co-culture assay used to validate the NY-ESO-1 TCR (c259) CD8+ NFAT-Luciferase Reporter Jurkat cell line.

Background

NY-ESO-1 (New York esophageal squamous cell carcinoma 1, also known as Cancer/testis antigen 1, or CTAG1B), is an important tumorigenic marker present in malignant cells. Normally expressed only in embryonic testis, this highly immunogenic protein is not usually found in normal tissues, but is re-expressed in multiple myeloma, non-small cell lung carcinoma, and breast and ovarian cancer, making it a promising candidate antigen for cancer immunotherapy. Several NY-ESO-1-directed therapies are being developed including cancer vaccines, anti-NY-ESO-1 adoptive cell therapy, and NY-ESO-1-specific TCR-T cell therapy in combination with checkpoint inhibitors.

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 NY-ESO-1-specific TCR cell evaluation.
- Use as a positive control in experiments evaluating NY-ESO-1 TCR cells.

Materials Provided

| Components | Format |
|-------------------------|--------------------------------------------------------------------------------------------------|
| 2 vials of frozen cells | Each vial contains 2×10^6 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 |
| NY-ESO-1 (157-165) Peptide | BPS Bioscience #78758 |
| APC MHC I Dextramer (HLA-A*02:01 SLLMWITQV) | Immudex #WB03247 |
| 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.

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% CO₂ 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% CO₂ incubator at 37°C until the cells are ready to passage.
5. Cells should be passaged before they reach a density of 2 x 10⁶ 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 10⁶ cells/ml, but no less than 0.2 x 10⁶ cells/ml, in Growth Medium 2T. The sub-cultivation ratio should maintain the cells between 0.2 x 10⁶ - 2 x 10⁶ 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 10⁶ 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 Assay Protocol:

1. Preparation of Antigenic Peptides
 - 1.1 Thaw the NY-ESO-1 (157-165) 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 5×10^5 /ml.
 - 2.2 Add 40 μ l of T2 cells into each well of a 96-well plate.
 - 2.3 Add 20 μ l of diluted peptide to the “peptide stimulated” APC wells.
 - 2.4 Add 20 μ l of Assay Medium 2D to the “unstimulated control” APC wells (for measuring basal luciferase activity).
3. Harvest the NY-ESO-1 TCR (c259) CD8⁺ NFAT Luciferase Reporter Jurkat cells from Growth Medium 2T by centrifugation and resuspend the cells in Assay Medium 2D at a density of 4×10^5 /ml.
4. Add 40 μ l of NY-ESO-1 TCR (c259) 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% CO₂ for 5-6 hours or overnight.
6. Add 100 μ l 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

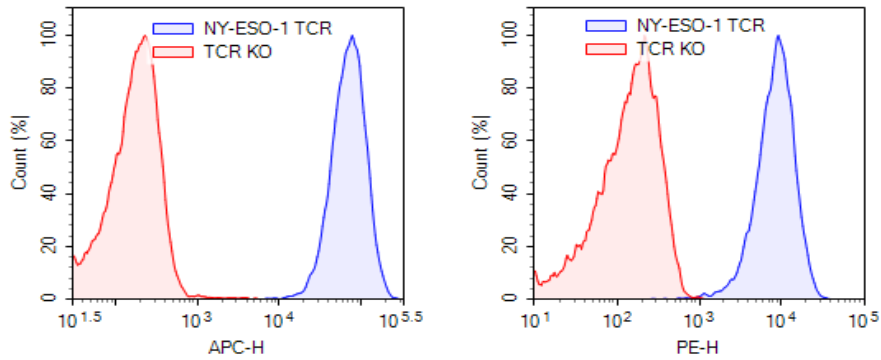


Figure 2: Expression of NY-ESO-1 TCR in NY-ESO-1 TCR (c259) CD8⁺ NFAT-Luciferase Reporter Jurkat cells.

Left: NY-ESO-1 TCR (c259) CD8⁺ NFAT Luciferase Reporter Jurkat cells were stained with APC-conjugated MHC-I Dextramer (HLA-A*02:01 SLLMWITQV; Immudex #WB03247) and analyzed by flow cytometry (blue). CD8⁺ TCR knockout NFAT Luciferase Reporter Jurkat cells were used as negative control (red).

Right: NY-ESO-1 TCR (c259) 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 a negative control (red).

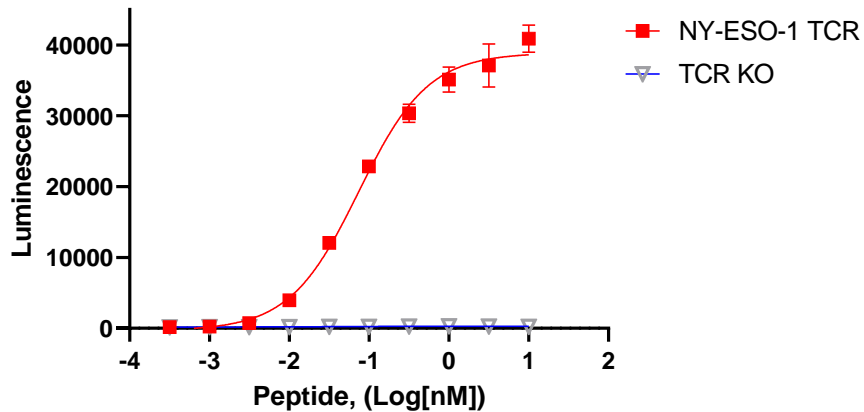


Figure 3: T Cell Activation using T2 cells as APC.

NY-ESO-1 TCR (c259) CD8⁺ NFAT Luciferase Reporter Jurkat cells were co-cultured for 6 hours with T2 cells loaded with various concentrations of NY-ESO-1 peptide (BPS Bioscience #78758). ONE-Step™ Luciferase was performed, and the results are shown as raw luminescence readings. CD8⁺ TCR-Knockout NFAT Luciferase reporter cell line, where no TCR is expressed, was 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

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

| <i>Products</i> | <i>Catalog #</i> | <i>Size</i> |
|-------------------------------------------------------------------|------------------|-------------------|
| 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 |
| CD8 ⁺ TCR KO NFAT Luciferase Reporter Jurkat Cell Line | 78757 | 2 vials |
| NY-ESO-1 Peptide | 78758 | 100 µl |