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Description

KRAS G12D TCR (Clone 9c) 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 KRAS (Kirsten sat sarcoma virus) G12D TCR (Clone 9c) using lentiviral transduction (with CD8a Lentivirus #78648 and KRAS G12D Specific TCR Lentivirus (Clone 9c) #78936). This TCR specifically recognizes the antigen KRAS G12D Peptide, amino acids 10-18 (GADGVGKSA, 9mer).

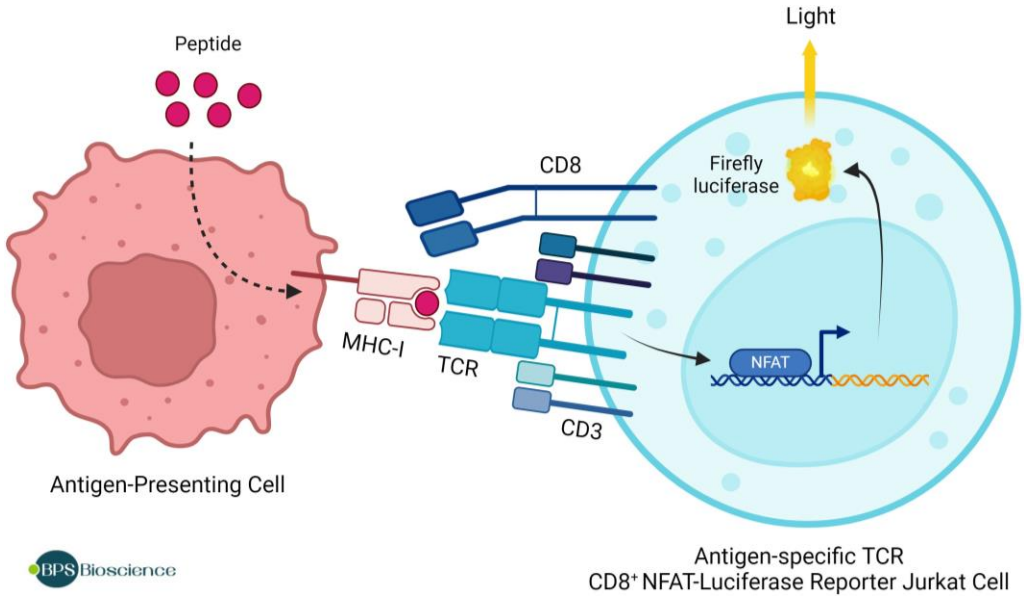

Figure 1: Illustration of the functional co-culture assay used to validate the KRAS G12D TCR (Clone 9c) CD8+ NFAT-Luciferase Reporter Jurkat Cell Line.

Background

KRAS (Kirsten rat sarcoma virus) are GTPase proteins. They cycle between a GDP-bound inactive state and a GTP-bound (active) form, in a process regulated by two accessory proteins: GEF (guanine exchange factors) and GAPs (GTPase activating proteins). Once activated KRAS can bind to its effectors and regulate multiple signaling pathways, such as the RAF (rapidly accelerated fibrosarcoma)-MEK (mitogen activated protein kinase)-ERK (extracellular regulated kinase) or the PI3K (phosphoinositide 3-kinase)-AKT (protein kinase B)-mTOR (mammalian target of rapamycin) signaling pathways. KRAS mutations account for about 85% of all RAS mutations and are considered one of the main drivers of human cancer, such as in PDAC (pancreatic ductal adenocarcinoma). One of the amino acids frequently mutated is glycine 12, with the most common form being G12D. Since KRAS are intracellular proteins, they are not amenable to CAR (chimeric antigen receptor)-T cell-based therapies, and the development of inhibitors has also proved challenging. One strategy involves the use of TCR (T cell receptor)-T cells, targeting this antigen. Specific TCR clones have been identified, with a KRAS G12D-specific TCR (clone 9c) preferentially being reactive against KRAS G12D peptide (10-18, 9mer), in comparison with KRAS G12D peptide (10-19, 10mer) and being unable to recognize the wild-type KRAS peptides. On the other hand, a KRAS G12D-specific TCR (clone 10) is preferentially reactive against KRAS G12D peptide (10-19, 10mer), in comparison with KRAS G12D peptide (10-18, 9mer) and it also does not recognize wild-type KRAS peptides. Results from a trial using a KRAS G12D HLA-C*08:02 restricted TCR demonstrated the potential of this approach for the treatment of PDAC. The use of neoantigen specific TCR-T cells, targeting single amino acid mutations, is thus an exciting and promising cancer therapy.

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 KRAS G12D-specific TCR cell evaluation.
- Use as a positive control in experiments evaluating KRAS G12D TCR 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
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2U	BPS Bioscience #82309
Assay Medium 2D	BPS Bioscience #78755
CD8+ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78757
HLA-C*08:02 K562 Cell line	BPS Bioscience #78974
KRAS G12D Peptide (10-18, 9mer)	BPS Bioscience #78967
KRAS G12D Peptide (10-19, 10mer)	BPS Bioscience #78969
PE anti-mouse TCR β chain Antibody	BioLegend #109207
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 (ATCC modification) supplemented with 10% FBS, and 1% Penicillin/Streptomycin.

Growth Medium 2U (BPS Bioscience #82309):
RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.25 mg/ml of Geneticin, 50 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 (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 2U.

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 2U. 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 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" wells.
- We recommend using CD8+ TCR Knockout NFAT-Luciferase Reporter Jurkat Cell Line as negative control.

1. Preparation of Antigenic Peptides

   1.1 Thaw the KRAS G12D peptide at Room Temperature (RT).

   1.2 Dilute the peptide with Assay Medium 2D to a concentration that is 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 HLA-C*08:02 K562 cells from Thaw Medium 2E and resuspend the cells in Assay Medium 2D at a density of 5 x 10^5/ml.

   2.2 Add 40 µl of HLA-C*08:02 K562 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 KRAS G12D TCR (Clone 9c) CD8+ NFAT-Luciferase Reporter Jurkat cells from Growth Medium 2U by centrifugation and resuspend the cells in Assay Medium 2D at a density of 4 x 10^5/ml.

4. Add 40 µl of KRAS G12D TCR (Clone 9c) CD8+ NFAT-Luciferase Reporter Jurkat cells into each well of the 96-well plate containing 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.

Validation Data

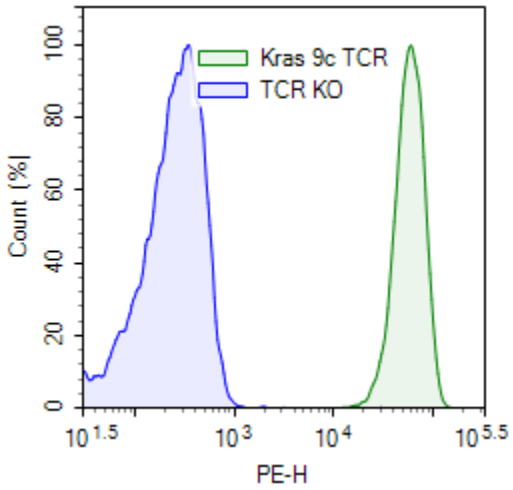

Figure 2: Flow cytometry analysis of the expression of KRAS G12D TCR in KRAS G12D TCR (Clone 9c) CD8+ NFAT-Luciferase Reporter Jurkat cells.
KRAS G12D TCR (clone#9c) CD8+ NFAT-Luciferase Reporter Jurkat cells (green) and CD8+ TCR Knockout NFAT-Luciferase Reporter Jurkat cells (blue) were stained with PE anti-mouse TCR β chain Antibody (BioLegend #109207) and analyzed by flow cytometry. The y axis represents the % of cells and the x axis the fluorophore intensity.

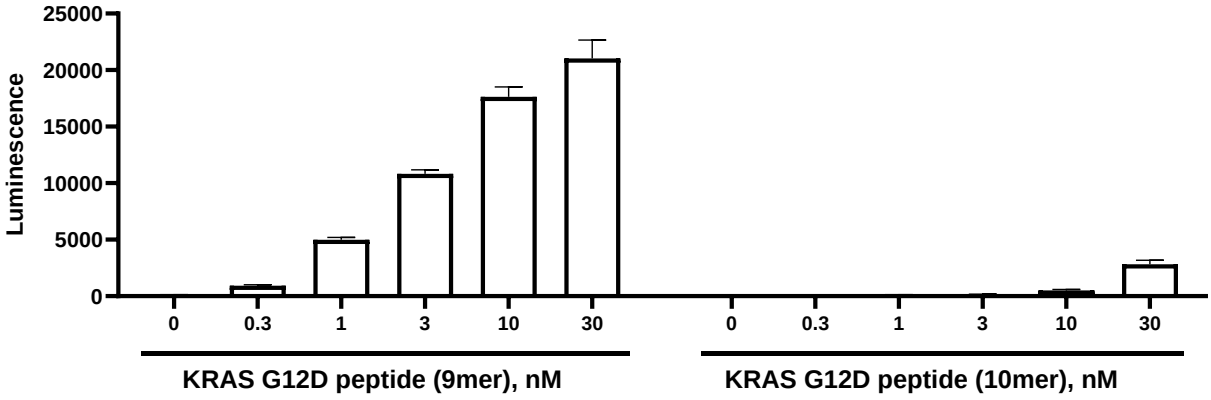

*Figure 3: KRAS G12D TCR (Clone 9c) CD8+ NFAT-Luciferase Reporter Jurkat Cell Line activation using HLA-C*08:02 K562 cells as APC.*
KRAS G12D TCR (Clone 9c) CD8+ NFAT-Luciferase Reporter Jurkat cells were co-cultured for 6 hours with HLA-C*08:02 K562 cells loaded with various concentrations of KRAS G12D peptide 9mer (#78967) and KRAS G12D peptide 10mer (#78969). Luciferase activity was measured with ONE-Step™ Luciferase Assay System, and the results are shown as raw luminescence readings. KRAS G12D TCR (Clone 9c) expressing cells preferentially recognize the KRAS G12D peptide 9mer.

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

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.

References

Eric T., *et al*., 2016 *N Engl J Med* 375:2255-2262.
Leidner R., *et al.*, 2022 *N Engl J Med* 386:2112-2119.

Related Products

Products	*Catalog #*	*Size*
KRAS G12D Specific TCR (Clone 9c) Lentivirus	78936	100 µl/2 x 500 µl
KRAS G12D peptide (10-18, 9mer)	78967	100 µl
KRAS G12D peptide (10-19, 10mer)	78969	100 µl
KRAS G12D TCR (Clone 10) CD8+ NFAT-Luciferase Reporter Jurkat Cell Line	82303	2 vials

Version 042324