Description

B2M (Beta-2-Microglobulin) has been genetically removed by CRISPR/Cas9 genome editing from NFAT Luciferase Reporter Jurkat cells. Expression of the firefly luciferase gene is driven by NFAT response elements located upstream of the minimal TATA promoter. Activation of the NFAT signaling pathway in these cells can be monitored by measuring luciferase activity.

Background

Beta-2-Microglobulin is a required component of Major Histocompatibility Complex (MHC) class 1 molecules, which present peptide fragments from within the cell to cytotoxic T cells as part of the adaptive immune system. The protein forms amyloid fibrils in some pathological conditions. A mutation in this gene drives hypercatabolic hypoproteinemia. B2M plays an essential role both in governing MHC class I molecule stability and in promoting antigen binding and presenting the antigen to CD3/TCR complex of CD8+ T cells.

NFAT (Nuclear factor of activated T-cells) 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 IL-2-3-4 and TNF-alpha) in T cells. NFAT is regulated by Ca2+ and the Ca2+/calmodulin-dependent serine phosphatase, calcineurin. NFAT is also activated by the T cell Receptor (TCR) in T cells.

Application

Study the consequences of B2M knock down or use as control in immune-oncology product development

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.

Media Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2R	BPS Bioscience #78411

Materials Required for Cellular Assay

Name	Ordering Information
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
NFAT Luciferase Jurkat Recombinant Cell Line	BPS Bioscience #60621
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white, clear-bottom plate	
Luminometer	

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, it is *highly recommended* to use these validated and optimized media from BPS Bioscience. 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 supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 2R (BPS Bioscience #78411):
RPMI 1640 medium supplemented with 10% FBS, plus 400 µg /ml of Hygromycin B

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

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 Hygromycin B)**.
   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 Hygromycin B).**

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 attachment and viability. Change medium to fresh Thaw Medium 2 **(no Hygromycin B)** 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 2R **(contains 400 µg/ml Hygromycin B)**.

Cell Passage

Dilute the cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml of Growth Medium 2R **(contains 400 µg/ml Hygromycin B)**. The sub-cultivation ratio is approximately 1:6 to 1:8 weekly or twice per week, so cells are maintained 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at a density of ~2 x 10^6 cells/ml.

2. Dispense 1 ml of cell aliquots into cryogenic vials. 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.

A. Validation Data

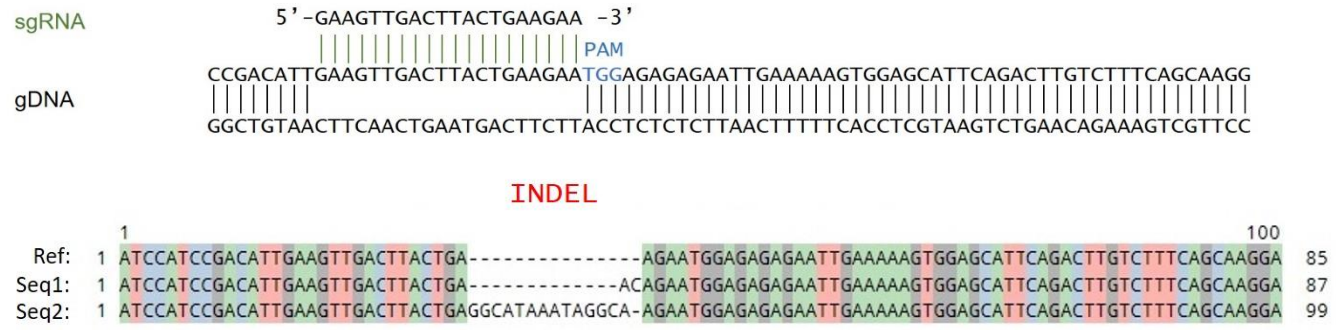

Figure 1: Genomic Sequencing of B2M in the B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line.

Genomic DNA from the B2M Knockout NFAT Luciferase Reporter Jurkat cells were isolated and sequenced. The PAM (Protospacer Adjacent Motif) is shown in blue, the sgRNA (synthetic guide RNA) and the Indels (Insertions / Deletions) in the two TRAC alleles are labeled, B2M genomic DNA labeled as Ref.

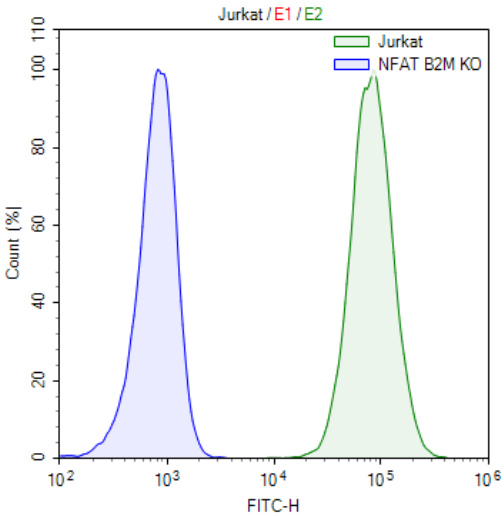

Figure 2: B2M Expression in B2M knockout NFAT Luciferase Reporter Jurkat cells.
Flow cytometry was performed using a PE-labeled anti-human B2M antibody (BioLegend #395703). Parental NFAT Luciferase Reporter Jurkat cells (red) were compared to B2M Knockout NFAT Luciferase Reporter Jurkat cells (blue). The Y-axis is the % cell number. The X-axis is the intensity of PE.

Assay Protocol to test NFAT-Luciferase Reporter Activity stimulated by anti-CD3 agonist

Medium required for the proposed assay:

Thaw Medium 2 (BPS Bioscience #60184):

RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin

Perform the experiment at least in triplicates.

1. Harvest the B2M Knockout NFAT Luciferase Reporter Jurkat cells and the positive control NFAT Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2. Dilute the cells to 5 x 10^5 cells/ml in the Thaw Medium (50µl/well, triplicates).

2. Prepare desired dilutions of the anti-CD3 antibody (e.g. 10 µg/ml) in 50 µl/well of the Thaw Medium, and mix with 50 µl of the B2M Knockout NFAT-Luciferase Reporter or control NFAT-Luciferase Reporter Jurkat cells. Plate 100 µl per well into a white clear-bottom 96-well plate in triplicates. The final cell density of the NFAT-Luciferase Reporter Jurkat cells is 25,000 cells per well.

3. Add 100 µl of Thaw Medium 2 to cell-free control wells (for determining background luminescence).

4. Incubate the plates at 37°C in a CO_2 incubator for 5-6 hours.

5. After the incubation, measure luciferase activity using the ONE-Step Luciferase Assay System: Add 100 µl of ONE-Step Luciferase Reagent per well and rock gently at room temperature for ~15 minutes. Measure the luminescence using a luminometer.

6. Data Analysis: Subtract the average background luminescence of the cell-free control wells from the luminescence reading of all wells.

7. The fold induction of NFAT luciferase reporter expression = average background-subtracted luminescence of antibody-treated well / average background-subtracted luminescence of untreated control wells.

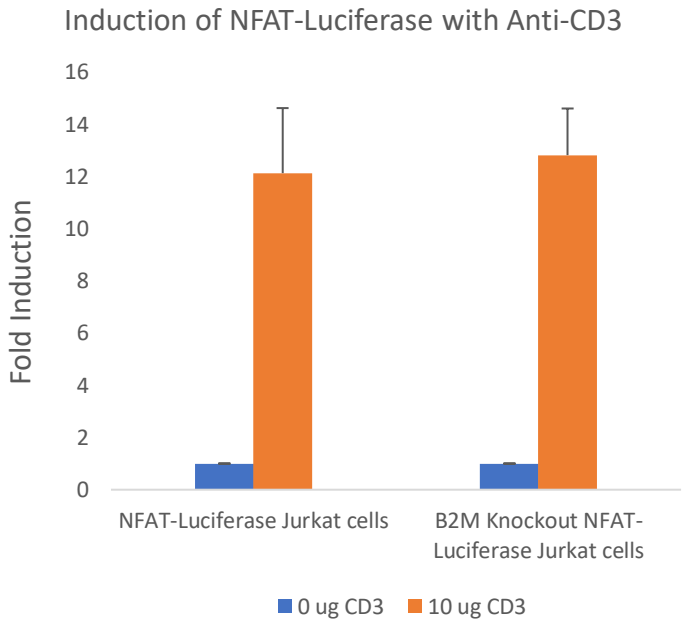

Figure 3. Stimulation of NFAT Luciferase activity with anti-CD3 Agonist Antibody.
Parental NFAT-Luciferase Jurkat cells and B2M knockout NFAT-Luciferase Jurkat cells were plated in triplicate at 25,000 cells/well into a clear-bottom, white 96-well plate. Each cell line was incubated with either 0 or 10 ug/ml anti-CD3 for 5 hours at 37°C. ONE-Step Luciferase reagent (BPS Bioscience, #60690) was added to the cells to measure NFAT activity. Background signal (no cells) was subtracted from all other values. The graph shows that NFAT signaling was not activated by the anti-CD3 agonist antibody in B2M knock-out cells.

Sequence
Human beta-2-microglobulin (B2M), mRNA, NCBI Reference Sequence: NM_004048.4, with the sgRNA targeting sequence underlined:

ATTCCTGAAGCTGACAGCATTCGGGCCGAGATGTCTCGCTCCGTGGCCTTAGCTGTGCTCGCGCTACTCTCTCTTTCTGGCCT
GGAGGCTATCCAGCGTACTCCAAAGATTCAGGTTTACTCACGTCATCCAGCAGAGAATGGAAAGTCAAATTTCCTGAATTGC
TATGTGTCTGGGTTTCATCCATCCGACATTGAAGTTGACTTACTGAAGAATGGAGAGAGAATTGAAAAAGTGGAGCATTCA
GACTTGTCTTTCAGCAAGGACTGGTCTTTCTATCTCTTGTACTACACTGAATTCACCCCCACTGAAAAAGATGAGTATGCCTG
CCGTGTGAACCATGTGACTTTGTCACAGCCCAAGATAGTTAAGTGGGATCGAGACATGTAAGCAGCATCATGGAGGTTTGA
AGATGCCGCATTTGGATTGGATGAATTCCAAATTCTGCTTGCTTGCTTTTTAATATTGATATGCTTATACACTTACACTTTATG
CACAAAATGTAGGGTTATAATAATGTTAACATGGACATGATCTTCTTTATAATTCTACTTTGAGTGCTGTCTCCATGTTTGAT
GTATCTGAGCAGGTTGCTCCACAGGTAGCTCTAGGAGGGCTGGCAACTTAGAGGTGGGGAGCAGAGAATTCTCTTATCCAA
CATCAACATCTTGGTCAGATTTGAACTCTTCAATCTCTTGCACTCAAAGCTTGTTAAGATAGTTAAGCGTGCATAAGTTAACT
TCCAATTTACATACTCTGCTTAGAATTTGGGGGAAAATTTAGAAATATAATTGACAGGATTATTGGAAATTTGTTATAATGAA
TGAAACATTTTGTCATATAAGATTCATATTTACTTCTTATACATTTGATAAAGTAAGGCATGGTTGTGGTTAATCTGGTTTATT
TTTGTTCCACAAGTTAAATAAATCATAAAACTTGA

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*
B2M (Human) CRISPR/Cas9 Lentivirus (Integrating)	78340	500 µl x2
B2M (Human) CRISPR/Cas9 Lentivirus (Non-Integrating)	78341	500 µl x2
NFAT Reporter Jurkat Cell Line	60621	2 vials
TCR Knockout Luciferase Reporter Jurkat Recombinant Cell Line	79887	2 vials
TCR/B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78364	2 vials