Description

Recombinant Jurkat T-cells expressing an enhanced GFP (eGFP) gene under the control of NFAT response elements located upstream of the minimal TATA promoter. Activation of the NFAT signaling pathway can be monitored by examining eGFP expression.

Background

The Nuclear Factor of Activated T-Cells (NFAT) family of transcription factors plays an important role in mediating the immune response. T-cell activation through the T-cell synapse results in calcium influx. Increased intracellular calcium levels activate the calcium-sensitive phosphatase Calcineurin, which rapidly dephosphorylates the serine-rich region (SRR) and SP-repeats in the amino termini of NFAT proteins. This results in a conformational change that exposes a nuclear localization signal (NLS) promoting NFAT nuclear translocation and inducing gene expression, including various cytokines (IL-2, IL-3, IL4, and TNF-alpha). Members of the NFAT family have been found in many tissue types, including the heart, skeletal muscle and brain. This reporter cell line is designed to monitor T-cell activation or inhibition through various checkpoint inhibitors. It can be used as a control or parental cell line to co-express various immune checkpoint inhibitors, such as PD1.

Application

- Screen for activators or inhibitors of NFAT signaling pathway
- Determine T-cell activation through T-cell receptor (TCR) or Chimeric Antigen Receptor (CAR)

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 2E	BPS Bioscience #79638

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Anti-CD3 Agonist Antibody	BPS Bioscience #71274
96-well tissue culture-treated white clear-bottom assay plate	
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
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℃ 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 (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2E (BPS Bioscience, #79638):
RPMI 1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.5 µg/ml Puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience, #60184):
RPMI 1640 medium (ATCC modification) 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 Puromycin).**
   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 Puromycin).**

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 **(no Puromycin)**, 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 2E **(contains Puromycin).**

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium 2E **(contains Puromycin).** The sub-cultivation ratio should maintain the cells 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

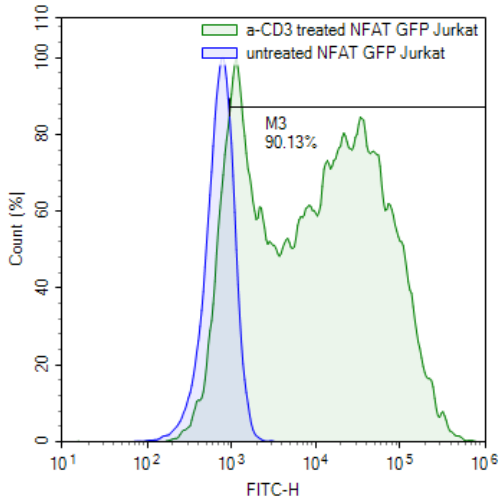

Figure 1: Expression of eGFP in NFAT Reporter (eGFP) Jurkat Cell Line. NFAT Reporter (eGFP) Jurkat cells (green) at a density of 2 x 10^6 cells/ml were stimulated with 10 ug/ml anti-CD3 Agonist Antibody (BPS Bioscience #71274) overnight. Unstimulated NFAT Reporter (eGFP) Jurkat cells (blue) were used as a negative control. Cells were then analyzed by flow cytometry. Y-axis is the % cell number. X-axis is the intensity of FITC.

References

- Clipstone NA, *et al.* (1992) *Nature* **357(6380)**: 695-697

- Lyakh L, *et al.* (1997) *Mol Cell Biol.* **17(5)**: 2475-2484

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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*
Anti-CD3 Agonist Antibody	71274	50 µg
NFAT Reporter (Luc) Jurkat Recombinant Cell Line	60621	2 vials
NFAT (eGFP) Reporter Lentivirus	79922	500 ul x 2
Thaw Medium 2	60184	100 ml
Growth Medium 2E	79638	500 ml