Description

LILRB4 NFAT-Luciferase Reporter Jurkat Cell Line are Jurkat cells stably expressing human LILRB4 (Leukocyte Immunoglobulin-Like Receptor Subfamily B Member 4, GenBank Accession #NM_001278426) and firefly luciferase under the control of NFAT response elements. Activation of NFAT in these cells can be monitored by measuring luciferase activity. This cell line was validated by flow cytometry for expression of LILRB4.

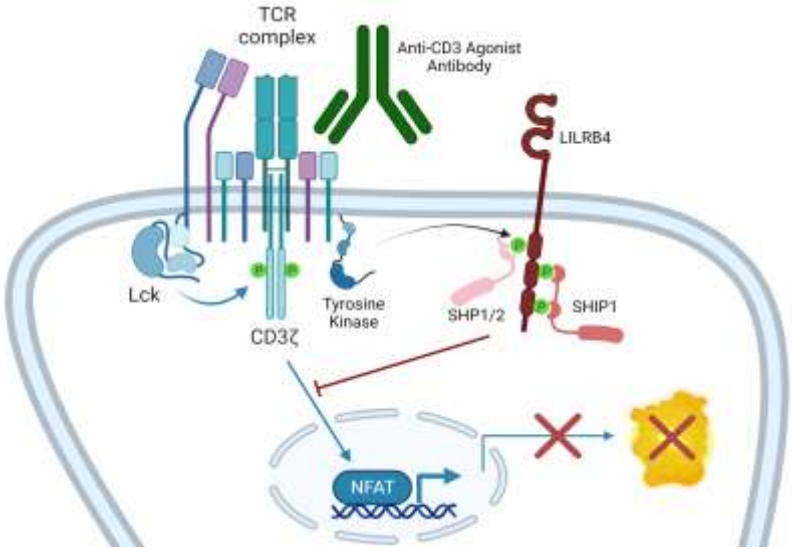

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Figure 1: Mechanism of action of LILRB4 NFAT Luciferase Reporter Jurkat Cell Line.
In response to TCR activation, LILRB4 is tyrosine phosphorylated by Src-family kinases such as Lck or Fyn, which triggers recruitment of SH2 domain-containing phosphatases, leading to attenuation of the T cell activation signals.

Background

Leukocyte Immunoglobulin-Like Receptor Subfamily B Member 4 (LILRB4, also known as B4, ILT3, LIR5, or CD85K) is a transmembrane immune checkpoint found in leukocytes (for example macrophages and dendritic cells) and involved in adaptive immunity. It binds MHC class I molecules at the surface of antigen-presenting cells, plays a role in antigen presentation, and transduces an inhibitory signal meant to down-regulate immune responses. Thus, it inhibits T cell proliferation, induces anergy, and regulates the development of immune tolerance.

The protein contains two extracellular immunoglobulin-like domains, a transmembrane domain, and three cytoplasmic immunoreceptor tyrosine-based inhibitory motifs (ITIMs) that allow it to be phosphorylated by cytosolic tyrosine kinases. Once phosphorylated, the ITIMs bind to SH2-domain containing phosphatases SHP-1/2 (SH2 domain-containing phosphatase 1 and 2) and SHIP-1 (Src homology 2 (SH2) domain containing inositol polyphosphate 5-phosphatase 1). SHP-1 dephosphorylates a wide spectrum of phospho-proteins involved in hematopoietic cell signaling whereas SHIP-1 dephosphorylates lipids at the plasma membrane and serves as a docking molecule. Both phosphatases are negative regulators of immune cell proliferation.

Differential LILRB4 expression levels have been observed in immune diseases such as Kawasaki disease, systemic lupus, and sepsis. Expression of the protein in Acute Myeloid Leukemia (AML) cells suppresses anti-tumor T cell activity. Because LILRB4 is highly expressed on AML cells but not on normal hematopoietic stem cells, it is considered a promising therapeutic target for CAR-T cells. It is also an immunotherapy target due to its role as an immune suppressive checkpoint. Indeed, blocking LILRB4 function is expected to improve anti-tumor immunity or to decrease auto-immunity and pathological inflammation.

Application
Control in experiments requiring LILR4 overexpression.

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 Used in Cellular Assays 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 2F	BPS Bioscience #79669

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 from 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, 1% Penicillin/Streptomycin

Growth Medium 2F (BPS Bioscience #79669):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml Geneticin, 0.5 µg/ml Puromycin Dihydrochloride.

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.
   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 2F.

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 2F. 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 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.

Validation Data

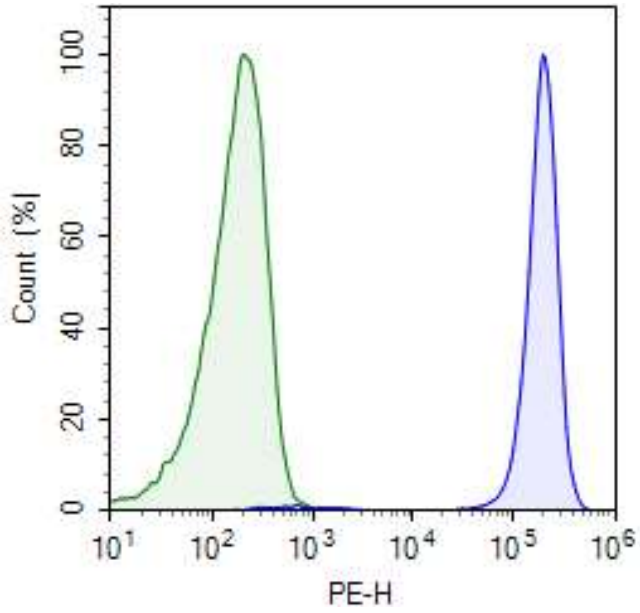

Figure 2: Expression of LILRB4 in LILRB4 NFAT-Luciferase Reporter Jurkat Cell Line by flow cytometry.
LILRB4 NFAT-Luciferase Reporter Jurkat Cells (blue) and parental NFAT-Luciferase Reporter Jurkat Cells (green) were stained with PE-labeled Anti- LILRB4 Antibody (BioLegend #333008) and analyzed by flow cytometry. Y-axis represents the % cell number. X-axis indicates the intensity of PE.

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

Sequence
Human LILRB4 sequence (accession number NM_001278426).

MIPTFTALLCLGLSLGPRTHMQAGPLPKPTLWAEPGSVISWGNSVTIWCQGTLEAREYRLDKEESPAPWDRQNPLEPKNKARFSI
PSMTEDYAGRYRCYYRSPVGWSQPSDPLELVMTGAYSKPTLSALPSPLVTSGKSVTLLCQSRSPMDTFLLIKERAAHPLLHLRSEH
GAQQHQAEFPMSPVTSVHGGTYRCFSSHGFSHYLLSHPSDPLELIVSGSLEGPRPSPTRSVSTAAGPEDQPLMPTGSVPHSGLRR
HWEVLIGVLVVSILLLSLLLFLLLQHWRQGKHRTLAQRQADFQRPPGAAEPEPKDGGLQRRSSPAADVQGENFCAAVKNTQPED
GVEMDTRQSPHDEDPQAVTYAKVKHSRPRREMASPPSPLSGEFLDTKDRQAEEDRQMDTEAAASEAPQDVTYARLHSFTLRQK
ATEPPPSQEGASPAEPSVYATLAIH

References
1. Deng M., *et al.*, 2018, *Nature* 562(7728): 605-609.
2. John S., *et al.*, 2018 *Mol Ther.*26(10): 2487-2495

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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*
LILRA1, Avi-His Tag	100245	100 µg
LILRB2, Avi-His Tag	100234	100 µg
LILRB1, Fc fusion, Biotin-labeled	79474	100 µg
TCR activator CHO cells	60539	2 vials