

Description

LILRB2 NFAT Luciferase Reporter Jurkat Cell Line is a Jurkat cell line that expresses human LILRB2 (leukocyte immunoglobulin-like receptor subfamily B member 2) and the firefly luciferase reporter under the control of NFAT response elements located upstream of the minimal TATA promoter.

LILRB2 expression was verified by flow cytometry, and this cell line was functionally validated with anti-LILRB2 and anti-HLA-G1 neutralizing antibodies in co-culture assays.

Background

LILRB2 (leukocyte immunoglobulin-like receptor subfamily B member 2), also known as ILT4, LIR2 or CD85D, is one of the 13 members of the LILR family of proteins characterized by associating with membrane adaptor proteins to trigger signals via ITIMS (immunoreceptor tyrosine-based inhibitory motifs) present in their long cytoplasmic tail. It is located on human chromosome 19q13.4, in the leukocyte receptor complex region, with other innate immune families of the Ig superfamily. It acts as an inhibitory receptor. It is expressed in monocytes, macrophages, and DCs (dendritic cells), and plays roles in synaptic plasticity, neurite growth and activation, and differentiation of immune cells. It has been shown to interact strongly with Amyloid β oligomers and has been implicated in AD (Alzheimer's disease), with anti-LILRB2 blocking antibodies showing promise in promoting microglia functions. It is found in the microenvironment of many types of tumors where it contributes to immune escape. Targeting LILRB2 is an active focus in antibody clinical research and may benefit patients suffering from LILRB2- related disorders.

Application

- Screen for inhibitors of LILRB2 signaling in a cellular model.
- Characterize the biological activity of LILRB2 and interaction with its ligands.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains $>1 \times 10^6$ 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 2J	BPS Bioscience #79812

Materials Required for Cellular Assay

Name	Ordering Information
HLA-G1 TCR Activator CHO Cell Line	BPS Bioscience #82458
NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60621
TCR Activator CHO Recombinant Cell Line	BPS Bioscience #60539
Thaw Medium 3	BPS Bioscience #60186
Anti-LILRB2 Neutralizing Antibody	Origene #TA389606
Anti-HLA-G1 Neutralizing Antibody	MyBioSource#MBS1581788
ONE-Step™ Luciferase Assay System	BPS Bioscience #60690
96-well tissue culture-treated white clear-bottom assay 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, 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% CO₂. 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):**

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2J (BPS Bioscience #79812):

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin, 0.5 mg/ml of Geneticin, and 1 µg/ml of puromycin.

Media Required for Functional Cellular Assay**Thaw Medium 2 (BPS Bioscience #60184):**

RPMI1640 medium (ATCC modification) supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Thaw Medium 3 (BPS Bioscience #60186):

Ham's F-12 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

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% 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 2J.

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, with Growth Medium 2J. The sub-cultivation ratio should maintain the cells between 0.2 x 10⁶ cells/ml and 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.

Validation Data

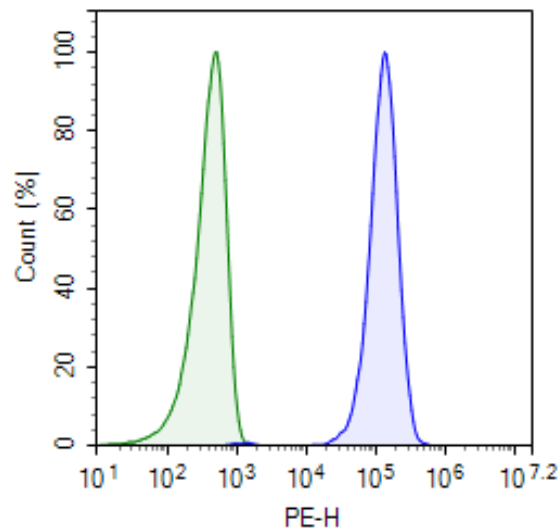


Figure 1. Cell surface expression of LILRB2 in LILRB2 NFAT Luciferase Reporter Jurkat Cell Line by flow cytometry.

LILRB2 NFAT Luciferase Reporter Jurkat cells (blue) and control NFAT Luciferase Reporter Jurkat cells (green) were stained with PE anti-human CD85d (ILT4) Antibody and analyzed by flow cytometry. Y-axis represents the cell count. X-axis indicates PE intensity.

Functional Validation

- This co-culture assay is designed to analyze the effect of the LILRB2/HLA-G1 interaction on Jurkat cell activation.
- 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 assays should include “Cell-Free Control”, “Untreated Control” and “Treated” conditions.
- The use of NFAT Luciferase Reporter Jurkat Cell Line (#60621) and TCR Activator CHO Recombinant Cell Line (#60539) as controls is recommended.

A. Testing of Anti-LILRB2 Antibody on a co-culture of LILRB2 NFAT Luciferase Reporter Jurkat Cell Line and HLA-G1 TCR Activator CHO Cell Line.

1. Seed HLA-G1 TCR Activator CHO cells at a density of 30,000 cells per well in 100 μ l of Thaw Medium 3 into a white, clear-bottom 96-well microplate. Leave a few wells empty to use as the “Cell-Free Control” (Background Signal).
2. Incubate the cells at 37°C in a CO₂ incubator overnight.
3. Prepare a serial dilution of anti-LILRB2 antibody in Thaw Medium 2 at 2x the final treatment concentration (50 μ l/well needed).

4. Harvest LILRB2 NFAT Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 4×10^5 cells/ml (50 μ l/well).
5. Preincubate LILRB2 NFAT Luciferase Reporter Jurkat cells (4×10^5 cells/ml) with the diluted anti-PD-1 antibody (1:1 in volume) for 30 minutes at 37°C (100 μ l mix/ well).
6. Remove the medium from HLA-G1 TCR Activator CHO cells and add 100 μ l of the LILRB2 NFAT Luciferase Reporter Jurkat cells/anti-LILRB2 antibody mixture to the “Treated” wells.

Note: Mix the LILRB2 NFAT Luciferase Reporter Jurkat cells with antibody thoroughly before adding to the CHO cells.

7. Add 50 μ l of LILRB2 NFAT Luciferase Reporter Jurkat cells (4×10^5 cells/ml) (no antibody) and 50 μ l of Thaw Medium 2 to the “Untreated Control” wells.
8. Add 100 μ l of Thaw Medium 2 to the “Cell-Free Control” wells (for determining background luminescence).
9. Incubate the plates at 37°C in a 5% CO₂ incubator overnight.
10. Add 100 μ l of ONE-Step™ Luciferase reagent per well.
11. Rock gently at room temperature (RT) for ~15 minutes.
12. Measure luminescence using a luminometer.
13. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

$$\text{Fold induction} = \frac{\text{luminescence treated wells} - \text{background}}{\text{luminescence untreated well} - \text{background}}$$

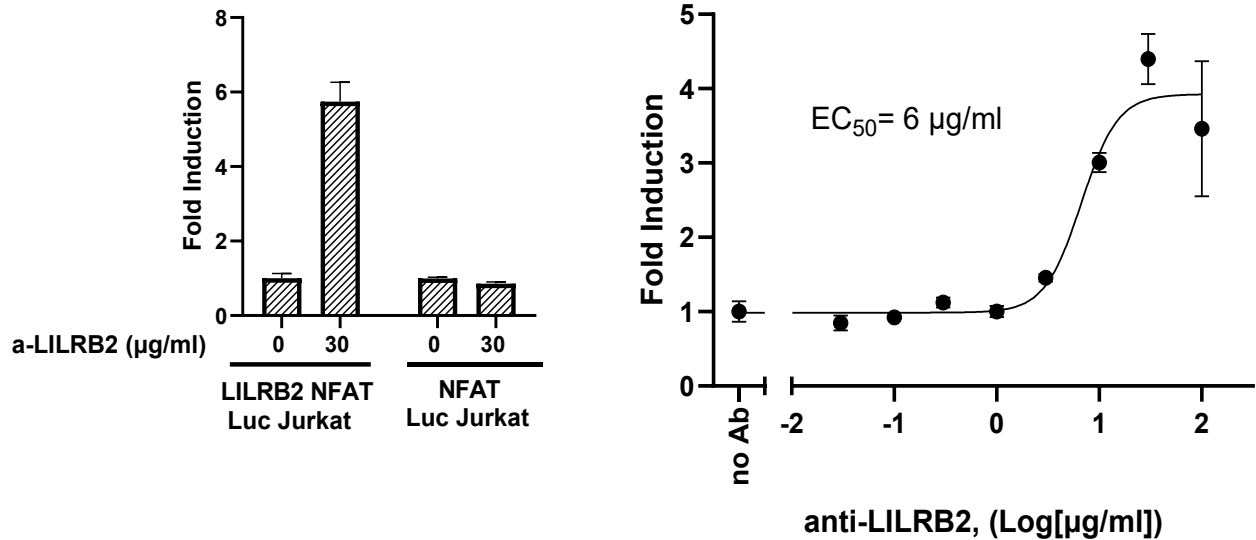


Figure 2. Effect of an anti-LILRB2 neutralizing antibody on NFAT activation in the LILRB2 NFAT Luciferase Reporter Jurkat Cell Line co-cultured with HLA-G1 TCR Activator CHO Cell Line.

Left. LILRB2 NFAT Luciferase Reporter Jurkat cells were co-cultured with HLA-G1 TCR Activator CHO cells as described in the protocol above in the presence or absence of an anti-LILRB2 neutralizing antibody. Addition of Anti-LILRB2 Neutralizing Antibody increased NFAT-induced luciferase reporter activity in LILRB2 NFAT Luciferase Reporter Jurkat cells, but not in NFAT Reporter Jurkat cells, when co-cultured with HLA-G1 TCR Activator CHO cells.

Right. LILRB2 NFAT Luciferase Reporter Jurkat cells were co-cultured with HLA-G1 TCR Activator CHO cells as described in the protocol above in the presence of increasing concentrations of Anti-LILRB2 Neutralizing Antibody, resulting in the dose-dependent activation of NFAT.

B. Testing of Anti-HLA-G1 Neutralizing Antibody on a co-culture of LILRB2 NFAT Luciferase Reporter Jurkat Cell Line and HLA-G1 TCR Activator CHO Cell Line.

1. Seed HLA-G1 TCR Activator CHO cells at a density of 30,000 cells per well in 100 µl of Thaw Medium 3 into a white, clear-bottom 96-well microplate. Leave a few wells empty to use as the "Cell-Free Control" (Background Signal).
2. Incubate the cells at 37°C in a CO₂ incubator overnight.
3. Prepare a serial dilution of anti-HLA-G1 antibody in Thaw Medium 2 at the final test concentration (50 µl/well needed).
4. Harvest LILRB2 NFAT Luciferase Reporter Jurkat cells by centrifugation and resuspend in Thaw Medium 2 at a density of 4 x 10⁵ cells/ml (50 µl/well).
5. Remove the medium from HLA-G1 TCR Activator CHO cells and add 50 µl of the diluted anti-HLA-G1 antibody to the "Treated" wells.
6. Add 50 µl of Thaw Medium 2 to the "Untreated Control" wells.

7. Incubate for 30 minutes at 37°C in a 5% CO₂ incubator.
8. Add 50 µl of LILRB2 NFAT Luciferase Reporter Jurkat cells (4 x 10⁵ cells/ml) to the “Treated” and “Untreated Control” wells.
9. Add 100 µl of Thaw Medium 2 to the “Cell-Free Control” wells (for determining background luminescence).
10. Incubate the plates at 37°C in a 5% CO₂ incubator overnight.
11. Add 100 µl of ONE-Step™ Luciferase reagent per well.
12. Rock gently at RT for ~15 minutes.
13. Measure luminescence using a luminometer.
14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

$$\text{Fold induction} = \frac{\text{luminescence treated wells} - \text{background}}{\text{luminescence untreated well} - \text{background}}$$

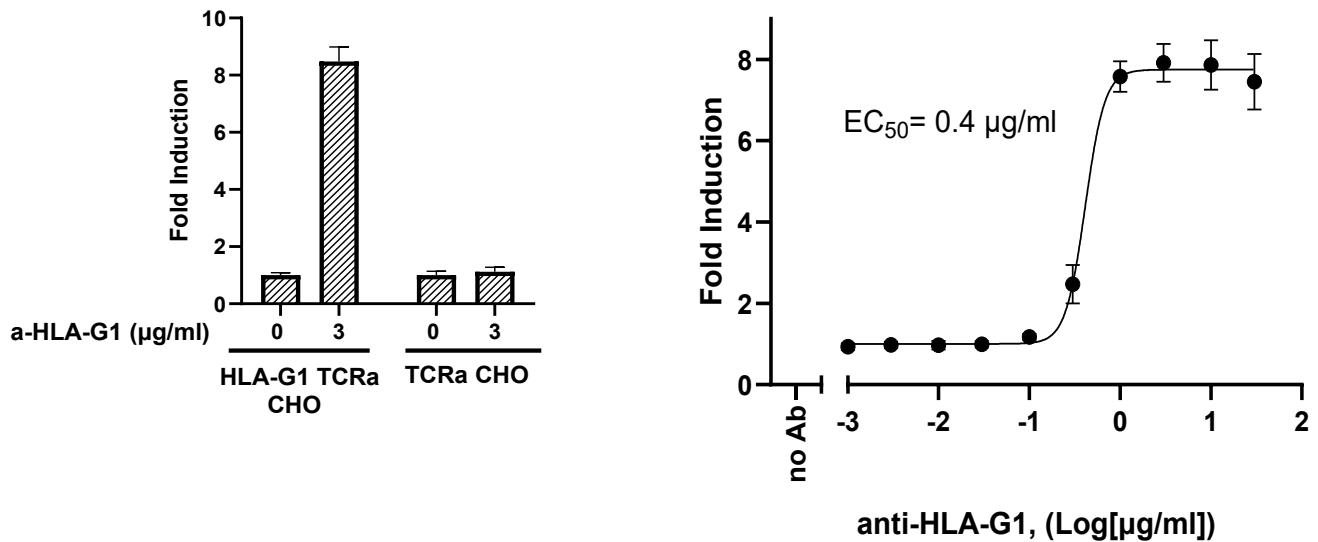


Figure 3. Effect of Anti-HLA-G1 Neutralizing Antibody on NFAT activation in the LILRB2 NFAT Luciferase Reporter Jurkat Cell Line co-cultured with HLA-G1 TCR Activator CHO Cell Line.

Left. LILRB2 NFAT Luciferase Reporter Jurkat cells were co-cultured with HLA-G1 TCR Activator CHO cells as described in the protocol above in the presence or absence of Anti-HLA-G1 Neutralizing Antibody. Addition of the antibody resulted in the activation of NFAT in LILRB2 NFAT Luciferase Reporter Jurkat cells.

Right. LILRB2 NFAT Luciferase Reporter Jurkat cells were co-cultured with HLA-G1 TCR Activator CHO cells as described in the protocol above in the presence of increasing concentrations Anti-HLA-G1 Neutralizing Antibody. Addition of the antibody resulted in the activation of NFAT and a dose-dependent increase in luciferase activity in the Jurkat cells co-cultured with HLA-G1 TCR Activator CHO cells.

Data shown is representative.

Sequence

Human LILRB2 sequence (accession number NP_001074447.2)

MTPIVTVLICLGLSLGPRTRVQTGTIPKPTLWAEPSVITQGSPVTLSCQGSLEAQEYRLYREKKSASWITRIRPELVKNGQFHIPSIT
WEHTGRYGCQYYSRARWSELSPLVLVMTGAYPKPTLSAQSPVVTSGGRVTLQCESQVAFGGFILCKEGEDHPQCLNSQPH
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QWRSSPAADAQEENLYAAVKDTQPEDGVEMDTRAAASEAPQDVTYAQLHSLTLRRKATEPPPSQEREPPEPSIYATLAIH

References

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Cao M., *et al.*, 2025 *Oncol Lett* 29(4):181.

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
HLA-G1 TCR activator CHO Cell Line	82458	2 vials
TCR Activator-CHO Recombinant Cell Line	60539	2 vials
NFAT Luciferase Reporter Jurkat Cell Line	60621	2 vials

Version 021226