Description

HLA-G1 TCR activator CHO Cell Line is a CHO-K1 cell line constitutively expressing human B2M (beta-2 microglobulin) and HLA-G1 (human leukocyte antigen) (NM_001363567.2) and an engineered TCR (T cell receptor) activator.

This cell line was functionally validated with anti-LILRB2 and anti-HLA-G1 neutralizing antibodies in co-culture assays.

Background

Human Leukocyte Antigen-G (HLA-G) is a non-classical MHC-I (major histocompatibility complex) heavy chain receptor, composed of HLA-G and β2-microglobulin (B2M). It has several isoforms, some of which are secreted while others are membrane bound. It is involved in fetal-maternal tolerance. In addition, it can serve as a marker for diabetic retinopathy and participate in immune evasion in cancer. The understanding of the association between HLA alleles and diseases will allow a refinement of therapeutic strategies.

Application

- Screen for activators or inhibitors of LILRB2/HLA-G1 signaling in a cellular model.
- Characterize the biological activity of HLA-G1 and interaction with its ligands.

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

CHO-K1 cells, Chinese Hamster Ovary, epithelial-like cells, adherent.

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 3	BPS Bioscience #60186
Growth Medium 3C	BPS Bioscience #79537

Materials Required for Cellular Assay

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
LILRB2 NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #83069
NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60621
TCR Activator CHO Recombinant Cell Line	BPS Bioscience #60539
Anti-LILRB2 Neutralizing Antibody	Origene #TA389606
Anti-HLA-G1 Neutralizing Antibody	MyBioSource#MBS1581788
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, 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 3 (BPS Bioscience #60186):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3C (BPS Bioscience #79537):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin and 5 µg/ml of Puromycin.

Media Required for Functional Cellular Assay

Thaw Medium 2 (BPS Bioscience #60535):
PMI1640 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 3.

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 3.

3. Transfer the resuspended cells to a T25 flask or T75 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 48-72 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 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 100% confluence. Switch to Growth Medium 3C for passage.

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS) without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3C and transfer to a tube.

3. Spin down cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3C.

4. Seed into new culture vessels at the recommended sub-cultivation ratio of 1:10 to 1:20 twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with PBS without Ca^{2+}/Mg^{2+}, and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3C and count the cells.

3. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 4°C Cell Freezing Medium (BPS Bioscience #79796) at ~2 x 10^6 cells/ml.

4. 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.

5. 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

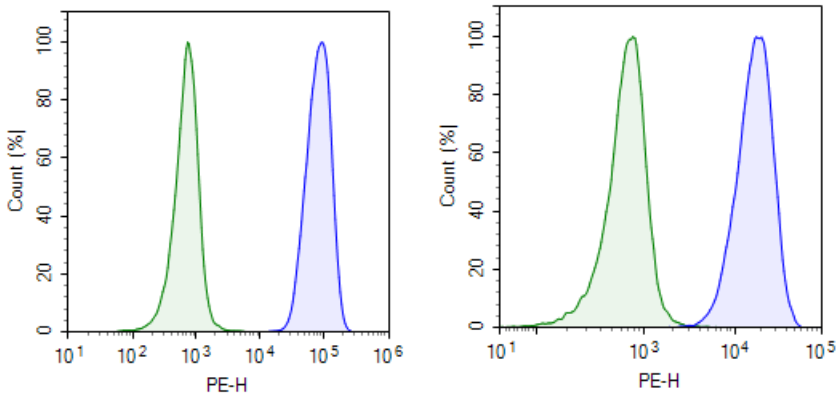

Figure 1. Cell surface expression of B2M and HLA-G1 in HLA-G1 TCR Activator CHO Cell Line by flow cytometry.
Left Panel: HLA-G1 TCR activator CHO cells (blue) and control TCR activator CHO cells (green) were stained with PE anti-human β2-microglubulin Antibody and analyzed by flow cytometry. Y-axis represents the cell count. X-axis indicates PE intensity.
Right Panel: HLA-G1 TCR activator CHO cells (blue) and control TCR activator CHO cells (green) were stained with PE anti-human HLA-G 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 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 CO_2 incubator overnight.

3. Prepare a serial dilution of anti-LILRB2 antibody in Thaw Medium 2 at 2x the final treatment 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 10^5 cells/ml (50 µl/well).

5. Preincubate LILRB2 NFAT Luciferase Reporter Jurkat cells (4 x 10^5 cells/ml) with the diluted anti-LILRB2 antibody (1:1 in volume) for 30 minutes at 37°C (100 µl mix/ well).

6. Remove the medium from HLA-G1 TCR Activator CHO cells and add 100 µl 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 µl of LILRB2 NFAT Luciferase Reporter Jurkat cells (4 x 10^5 cells/ml) (no antibody) and 50 µl of Thaw Medium 2 to the "Untreated Control" wells.

8. Add 100 µl of Thaw Medium 2 to the "Cell-Free Control" wells (for determining background luminescence).

9. Incubate the plates at 37°C in a 5% CO_2 incubator overnight.

10. Add 100 µl 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.

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

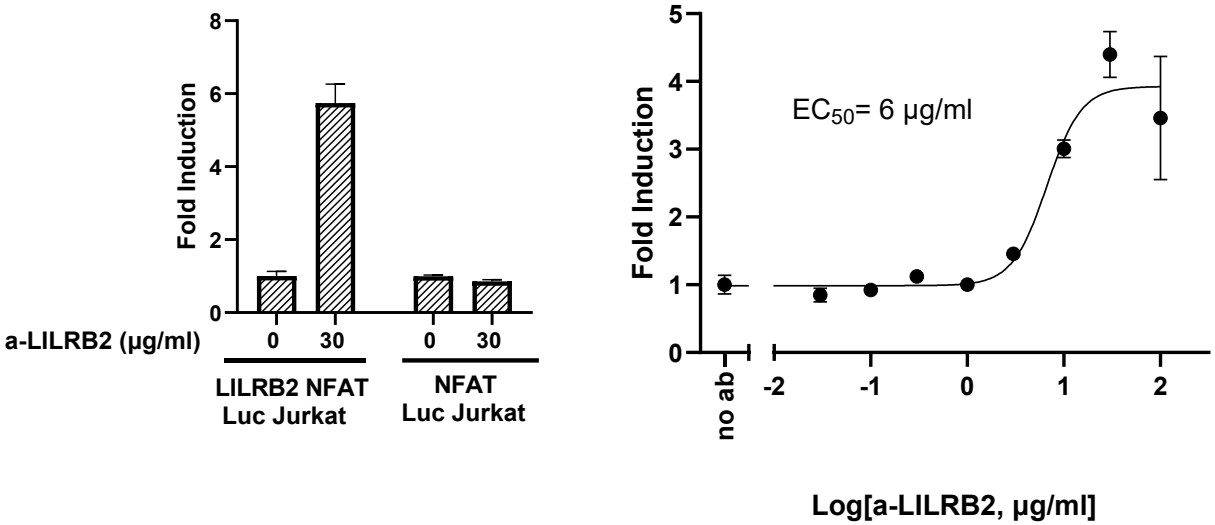

Figure 2. Effect of an anti-LILRB2 neutralizing antibody on NFAT activation in the LILRB2 NFAT Luciferase Reporter Jurkat Cell Line co-cultured with HLA-G1 TCR Activator CHO Cell Line.
Left. LILRB2 NFAT Luciferase Reporter Jurkat cells were co-cultured with HLA-G1 TCR Activator CHO cells as described 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 CO_2 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 10^5 cells/ml (50 µl/well).

5. Remove the medium from HLA-G1 TCR Activator CHO cells and add 50 µl of the anti-HLA-G1 antibody dilution 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% CO_2 incubator.

8. Add 50 µl of LILRB2 NFAT Luciferase Reporter Jurkat cells (4 x 10^5 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% CO_2 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.

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

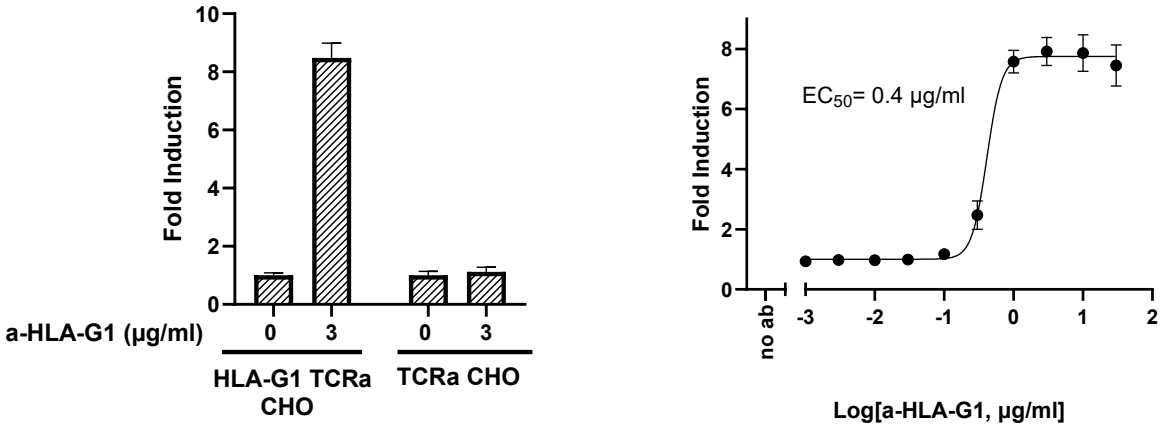

Figure 3. Effect of Anti-HLA-G1 Neutralizing Antibody on NFAT activation in LILRB2 NFAT Luciferase Reporter Jurkat Cell Line co-cultured with HLA-G1 TCR Activator CHO Cell Line.
Left. LILRB2 NFAT Luciferase Reporter Jurkat cells were co-cultured with HLA-G1 TCR Activator CHO cells as described in the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

References

Taghvaei-Bijandi E., *et al.*, 2023 *Iran J Immunol* 20(2):202-210.
Barbaro G., *et al.*, 2023 *Int J Mol Sci.* 24(3):2557.

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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

Products	*Catalog #*	*Size*
B2M HLA-G1 Lentivirus	82445	500 µl x 2
TCR Knockout Jurkat Cell Line	78539	2 vials
B2M Knockout THP-1 Cell Line	78389	2 vials

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