Description

Recombinant Jurkat cell line expressing firefly luciferase under the control of an IL-2-responsive promoter, and with constitutive expression of human KIR3DL3 (Killer Cell Immunoglobulin Like Receptor, Three Ig Domains and Long Cytoplasmic Tail 3; GenBank accession #BC143802.1 corresponding to KIR3DL3*00402 allele). HHLA2 (B7-H7) mediates an immune-stimulatory signal via TMIGD2 (Transmembrane and immunoglobulin domain containing 2; CD28H) in naïve T cells while it delivers an immune-inhibitory signal through KIR3DL3 in activated T cells and Natural Killer (NK) cells.

Application

- Characterize the biological activity of KIR3DL3 interaction with B7-H7
- Screen for anti-B7-H7 antibodies in co-culture with B7-H7-expressing cells

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)

Host Cell

Jurkat cells (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 2P	BPS Bioscience #78354

Materials Required for Cellular Assay

Name	Ordering Information
Anti-HHLA2(B7-H7) antibody[1]	
Growth Medium 2P	BPS Bioscience #78354
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
B7-H7(HHLA2)/TCR Activator CHO Cell Line	BPS Bioscience #78321
Anti-CD28 agonist antibody	BPS Bioscience #100186
96-well tissue culture treated, white clear-bottom assay plate	Corning #3610
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°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):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2P (BPS Bioscience #78354):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 0.25 µg/ml of Puromycin and 1 mg/ml Geneticin.

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 Geneticin or 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 Geneticin or 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 attachment and viability. Change medium to fresh Thaw Medium 2 **(no Geneticin or 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 2P **(contains Geneticin and Puromycin)**.

Cell Passage

Dilute the cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml of Growth Medium 2P **(contains Geneticin and Puromycin)**. Cells should be 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 106 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.

Assay Performance

The following assays are designed for a 96-well format. To perform the assay in different tissue culture formats, cell number and reagent volume should be scaled appropriately.

Assay Medium: Thaw Medium 2 (BPS Bioscience #60184).

A. Evaluation of an anti-HHLA2(B7-H7) antibody by monitoring KIR3DL3/IL-2 Luciferase Reporter Jurkat Cell Line in a co-culture assay with B7-H7(HHLA2)/TCR Activator CHO Cell Line.

1. In a white clear-bottom 96- well plate, seed B7-H7(HHLA2)/TCR Activator CHO cells at 2 x 10^4 cells/well in the Assay Medium. Allow the cells attach overnight by incubating at 37°C with 5% CO_2. Cells should be around 80-90% confluence the next day before addition of the Jurkat reporter cell line.
2. Culture KIR3DL3/IL-2 Luciferase Reporter Jurkat cells so that they are at 1 ~ 2 x 10^6 cells/ml on the day of the experiment.
3. The next day, carefully remove the medium from each well of the CHO cells plate, and add anti-HHLA2(B7-H7) antibody[1] at 2X concentrations, diluted in 50 µl of Assay Medium (Thaw Medium 2). Incubate the plate for one hour at 37°C with 5% CO_2.
4. After one hour of pre-incubation with the antibody, add KIR3DL3-IL2-Jurkat reporter cells at 2-4 x 10^4 cells/well in 50 µl of Assay Medium supplemented with 1 µg/ml of anti-CD28 TCR-activating antibody (BPS Bioscience, #100186). Incubate the plate for approximately 5-6 hours at 37°C with 5% CO_2. The final volume is 100 µl.
5. Prepare the ONE-Step™ Luciferase Assay reagent per recommended instructions. Add 100 µl of ONE-Step™ Luciferase reagent per well. Incubate at room temperature for ~15 to 30 minutes and measure luminescence using a luminometer.

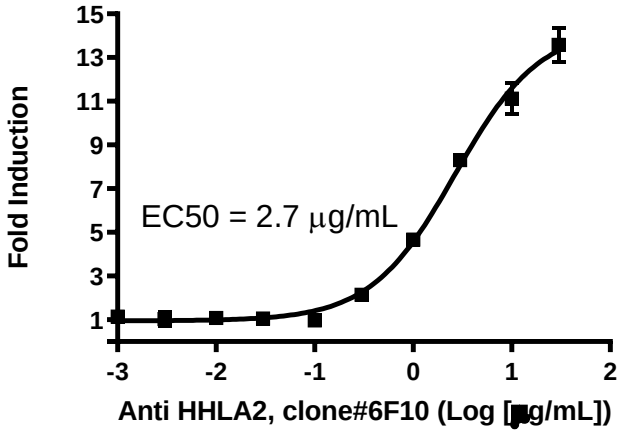

Figure 1. Dose-dependent Luciferase activity in KIR3DL3/IL-2 Luciferase Reporter Jurkat Cell Line in response to anti-HHLA2(B7-H7) antibody 6F10 when co-cultured with B7-H7(HHLA2)/TCR Activator CHO Cell Line in the presence of TCR agonist anti-CD28. B7-H7(HHLA2) delivers an immune-inhibitory signal through KIR3DL3 in anti-CD28 activated T cells, which is counteracted by the anti-HHLA2(B7-H7) antibody. Signal induction was calculated based on the signal from the well containing no anti-HHLA2 antibody

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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.

References

1. Bahatt RS, et. al., *Cancer Immunol Res*. 2021 Feb;**9(2):**156-169.

Related Products

Products	*Catalog #*	*Size*
B7-H7 (HHLA2)/TCR Activator CHO Cell line	78321	2 vials
TMIGD2(CD28H)/NFAT (Luciferase) - Jurkat	78323	2 vials
Anti-CD28 agonist antibody (humanized)	100186-1	50 µg
Anti-CD28 Agonist Antibody	100182-1	50 µg
ONE-Step™ Luciferase Assay System	60690	Multiple sizes
Thaw Medium 2	60184	100 ml
Growth Medium 2P	78354	500 ml
B7-H7, Fc-Fusion, Avi-Tag	79365	100 µg
B7-H7, Fc-Fusion, Avi-Tag, Biotin-Labeled	79366-1	250 µg