Description

Recombinant CHO-K1 cells stably expressing human B7-H7 (also known as HHLA2: HERV-H LTR-associating protein 2, GenBank accession #NM_007072) and an engineered T cell receptor (TCR) activator. B7-H7 mediates an immune-stimulatory signal via TMIGD2 (Transmembrane and immunoglobulin domain containing 2) in naïve T cells while delivering an immune-inhibitory signal through KIR3DL3 in activated T cells and Natural Killer (NK) cells.

Application

- Characterize the biological activity of KIR3DL3 or TMIGD2 interactions with B7-H7
- Screen for B7-H7 antibodies

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

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 3A	BPS Bioscience #60188

Materials Required for Cellular Assay

Name	Ordering Information
Anti-HHLA2(B7-H7) Antibody[1]	Bahatt RS, 2021 (see references)
Growth Medium 3A	BPS Bioscience #60188
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
KIR3DL3/IL-2 Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78322
TMIGD2/NFAT Luciferase Reporter Jurkat Cell Line	BPS Bioscience #78323
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	

BPS Bioscience ● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

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 3 (BPS Bioscience #60186):
F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin

Growth Medium 3A (BPS Bioscience #60188):
F-12K Medium (Kaighn's Modification of Ham's F-12 Medium) supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin and 500 µg/ml of Hygromycin.

Media Required for Cellular Assay

Thaw Medium 2 (BPS Bioscience #60184):
RPMI1650 medium 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 (**no Hygromycin or Geneticin**).
   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 (**no Hygromycin or Geneticin**).

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 3 (**no Hygromycin or Geneticin**) 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 3A **(contains Hygromycin and Geneticin).**

Cell Passage

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3A and transfer to a tube. Spin down cells at 300 x g for 5 minutes, remove the medium and resuspend the cells in Growth Medium 3A **(contains Geneticin and Hygromycin).** Seed into new culture vessels at the desired sub-cultivation ratio of 1:10 once or twice a week.

Cell Freezing

1. Aspirate the medium, wash the cells with phosphate buffered saline (PBS), and detach the cells from the culture vessel with 0.25% Trypsin/EDTA.

2. Once the cells have detached, add Growth Medium 3A 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 Freezing Medium (BPS Bioscience #79796, or 10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml.

4. 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.

5. 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. Functional characterization of B7-H7 (HHLA2)/TCR Activator CHO Cell Line.

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 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 Growth Medium 3A. Allow the cells to 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 cell line. Prepare medium-only wells to measure background signal (blank).

2. Culture the 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, prepare the anti-HHLA2(B7-H7) antibody at concentrations 2-fold higher than the desired final concentrations, using 50 µl/well of Thaw Medium 2.

4. Carefully remove the medium from each well of the CHO cells plate and add 50 µl/well of the diluted antibody. Incubate the plate for one hour at 37°C with 5% CO_2.

5. After one hour of pre-incubation with the antibody, add KIR3DL3/IL2 Luciferase Reporter Jurkat cells at 2-4 x 10^4 cells/well in 50 µl of Thaw Medium 2 supplemented with 1 µg/ml of anti-CD28 TCR-activating antibody (BPS Bioscience #100186).

6. Incubate the plate for approximately 5-6 hours at 37°C with 5% CO_2. The final volume is 100 µl.

7. Prepare the ONE-Step™ Luciferase Assay reagent per recommended instructions.
   - Thaw Luciferase Reagents ahead of time. Equilibrate Component A at room temperature and mix well before use.
   - Calculate the amount of Luciferase Reagent needed for the experiment (Component A + Component B). Immediately before use, add 1 volume of Component B to 99 volumes of Component A (1:100 ratio) and mix well. Avoid exposure to excessive light. Store the remaining Component A and Component B separately at -20°C.

   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.

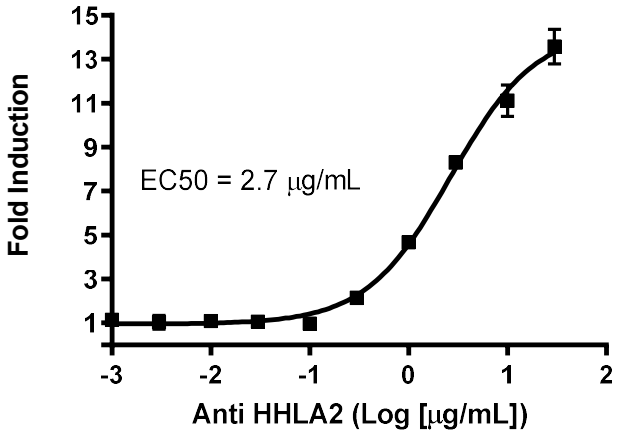

Figure 1. Activation of KIR3DL3/IL-2 Luciferase Reporter Jurkat cells in response to increasing doses of anti-HHLA2 antibody when co-cultured with B7-H7 (HHLA2)/TCR Activator CHO cells in the presence of TCR agonist anti-CD28.
B7-H7(HHLA2) delivers an immune-inhibitory signal through KIR3DL3 in anti-CD28 activated T cells, which is counteracted by the anti-HHLA2 antibody. Signal induction was calculated based on the signal from the well containing no anti-HHLA2 antibody. The antibody used in this experiment has been described in Bahatt RS, *et al.*, (2021).

b. Evaluation of a neutralizing anti-HHLA2 antibody by monitoring TMIGD2/NFAT 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 Growth Medium 3A. Allow the cells to 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 reporter cell line. Prepare medium-only wells to measure background signal (blank).

2. Culture TMIGD2/NFAT Luciferase Reporter Jurkat cells so that they are at ~ 1 to 2 x 10^6 cells/ml on the day of the experiment.

3. The next day, carefully remove the medium from the B7-H7 (HHLA2)/TCR Activator CHO plate and add 50 µl of anti-HHLA2 antibody at concentrations that are 2-fold higher than the desired final concentrations, diluted in 50 µl of Thaw Medium 2. Add medium only (no antibody) to the control wells.

4. Incubate the plate for one hour at 37°C with 5% CO_2.

5. After one hour of pre-incubation with the antibody, add TMIGD2/NFAT Luciferase Reporter Jurkat cells at 4-5 x 10^4 cells/well in 50 µl of Thaw Medium 2. Add the same number of TMIGD2/NFAT Luciferase Reporter Jurkat cells to the control wells.

6. Incubate the plate for approximately 3-6 hours at 37°C with 5% CO_2. The final volume is 100 µl.

7. Prepare the ONE-Step™ Luciferase Assay reagent per recommended instructions.
   - Thaw Luciferase Reagents ahead of time. Equilibrate Component A at room temperature and mix well before use.
   - Calculate the amount of Luciferase Reagent needed for the experiment (Component A + Component B). Immediately before use, add 1 volume of Component B to 99 volumes of Component A (1:100 ratio) and mix well. Avoid exposing to excessive light. Store the remaining Component A and Component B separately at -20°C.

   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.

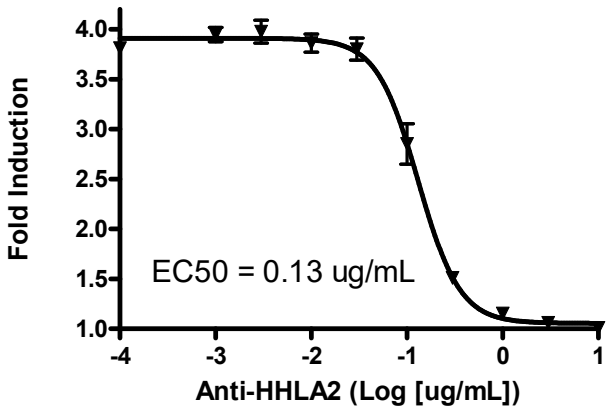

Figure 2. TMIGD2/NFAT Luciferase Reporter Jurkat Cell Line activity in response to increasing doses of anti-HHLA2 antibody when co-cultured with B7-H7 (HHLA2)/TCR Activator CHO cells.
B7-H7 mediates an immune-stimulatory signal via TMIGD2 that is inhibited by the anti-HHLA2 antibody. Signal induction was calculated based on the signal from the well containing no anti-HHLA2 antibody. The antibody used in this experiment has been described in Bahatt RS, *et al.*, (2021).

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Troubleshooting Guide
Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please emailsupport@bpsbioscience.com.

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

Related Products

Products	*Catalog #*	*Size*
TMIGD2/NFAT Luciferase Reporter Jurkat Cell Line	78323	2 vials
KIR3DL3/IL-2 Luciferase Reporter Jurkat Cell Line	78322	2 vials
Anti-CD28 Agonist Antibody (Humanized)	100186	50 µg/100 µg
Anti-CD28 Agonist Antibody	100182	50 µg/100 µg
B7-H7, Fc-Fusion, Avi-Tag	79365	100 µg
B7-H7, Fc-Fusion, Avi-Tag, Biotin-Labeled	79366	25 µg/50 µg