Description

Recombinant Jurkat cell line expressing firefly luciferase under the control of an NFAT response element, and with constitutive expression of human TMIGD2 (Transmembrane and immunoglobulin domain containing 2; CD28H; NM_144615). Expression of the firefly luciferase gene is driven by NFAT response elements located upstream of the minimal TATA promoter. Activation of the NFAT signaling pathway in these cells can be monitored by measuring luciferase activity.

Background

HHLA2 (B7-H7) mediates an immune-stimulatory signal via TMIGD2 in naïve T cells while it delivers an immune-inhibitory signal through KIR3DL3 (Killer Cell Immunoglobulin Like Receptor, Three Ig Domains and Long Cytoplasmic Tail 3) in activated T cells and Natural Killer (NK) cells.

Application

- Characterize the biological activity of TMIGD2 interaction with B7-H7
- Screen for B7-H7 antibodies in co-culture with B7-H7-expressing cells

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)

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.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

Materials Required for Cellular Assay

Name	Ordering Information
Anti-HHLA2(B7-H7) antibody [1]	
Growth Medium 2A	BPS Bioscience #60190
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
B7-H7(HHLA2)/TCR Activator CHO Cell Line	BPS Bioscience #78321
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 2A (BPS Bioscience #60190):
RPMI1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 200 µg/ml of Hygromycin 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 Hygromycin)**.
   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 Hygromycin)**.
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 **(no Geneticin or Hygromycin)**, 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 2A **(contains Geneticin and Hygromycin)**.

Cell Passage
Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, at no less than 0.2 x 10^6 cells/ml of Growth Medium 2A **(contains Geneticin and Hygromycin)**. The sub-cultivation ratio should maintain the cells between 0.2 x 10^6 cells/ml and 2 x 10^6 cells/ml.

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.

A. Evaluation of a neutralizing anti-HHLA2(B7-H7) antibody by monitoring TMIGD2/NFAT Luciferase Reporter Jurkat cell line in a co-culture assay with B7-H7/TCR activator CHO recombinant cell line

1. In a white clear-bottom 96-well plate, seed B7-H7/TCRa - CHO cells at 2 x 10^4 cells/well in Thaw Medium 2. 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 TMIGD2/NFAT Reporter Jurkat cell line.
2. Culture TMIGD2/NFAT Luciferase Reporter Jurkat cell line so that they reach a density of 1 to 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 B7-H7/TCRa CHO plate, and add 50 µl/well of anti HHLA2(B7-H7) antibody[1] at 2-fold concentrations (compared to the final desired concentrations) diluted in 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 TMIGD2/NFAT Luciferase Reporter Jurkat cells at 4-5 x 10^4 cells/well in 50 µl of Assay Medium (Thaw Medium 2). Incubate the plate for approximately 5-6 hours at 37°C with 5% CO_2. The final volume is 100 µl.
5. Prepare 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.

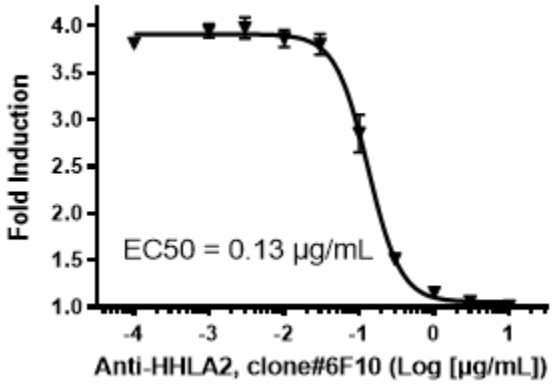

Figure 1. Dose response of NFAT reporter activity in response to anti HHLA2(B7-H7) antibody 6F10 when co-cultured with B7-H7/TCRa - CHO cells. TMIGD2/NFAT Luciferase Reporter Jurkat cells were added to *B7-H7/TCRa - CHO* cells pre-incubated with increasing concentrations of neutralizing anti HHLA2(B7-H7) antibody and TMIGD2-activated luciferase activity was measured using the OneStep™ Luciferase Assay System reagent (BPS Bioscience #60690). B7-H7(HHLA2) delivers an immune-activating signal through TMIGD2 in T cells, which is counteracted by the anti-HHLA2(B7-H7) antibody. Signal induction was calculated based on the signal from the well containing TCRa-CHO cells (BPS Bioscience #60539)

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

Related Products

Products	*Catalog #*	*Size*
B7-H7 (HHLA2)/TCR Activator CHO Cell line	78321	2 vials
KIR3DL3/IL-2 Luciferase Reporter Jurkat Cell Line	78322	2 vials
Anti-CD28 Agonist Antibody (Humanized)	100186	50 µg
Anti-CD28 Agonist Antibody	100182	50 µg
ONE-Step™ Luciferase Assay System	60690	Multiple sizes
Thaw Medium 2	60184	100 ml
Growth Medium 2A	60190	500 ml
B7-H7, Fc-Fusion, Avi-Tag	79365	100 µg
B7-H7, Fc-Fusion, Avi-Tag, Biotin-Labeled	79366	250 µg