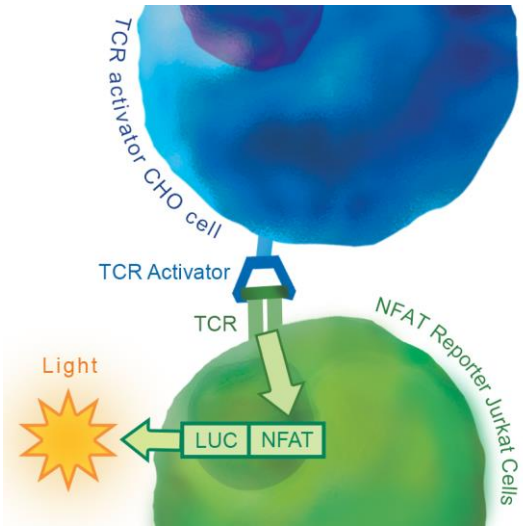
Description

Recombinant CHO cell line stably expressing an engineered, membrane-bound T cell receptor (TCR) activator. The cell line has been functionally validated in a co-culture assay for activation of the NFAT Reporter Jurkat cells and was shown to induce the proliferation of human Peripheral Blood Mononuclear Cells (PBMC).



Background

The T Cell Receptor (TCR) is a heterodimer protein found at the surface of T cells, which is stimulated upon binding to MHC/peptide complexes and triggers a signaling cascade that leads to the activation of transcription factors involved in the upregulation and secretion of cytokines, T cell proliferation, and cell differentiation into effector and memory cells.

When co-cultured with T cells, the engineered TCR activator cells stimulate the TCR on T cells, leading to activation of downstream signaling pathways and biological responses such as proliferation.

Application

- Activate T cells in co-culture assays
- Use as control cell line for TCR activator/PD-L1 CHO cell line (BPS Bioscience #60536) and other similar cell lines

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)

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 3B	BPS Bioscience #79529

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 supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 3B (BPS Bioscience #79529):
F-12K medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 500 µg/ml of Hygromycin B.

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)**.
   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)**.

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 24 hours of culture, check for cell attachment and viability. Change medium to fresh Thaw Medium 3 **(no 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 are fully confluent. At first passage and subsequent passages, use Growth Medium 3B **(contains Hygromycin)**.

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 3B 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 3B **(contains Hygromycin)**. Seed into new culture vessels at the desired sub-cultivation ratio of 1:6 to 1:8 weekly or twice per 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 3B 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. Validation Data

The functionality of the cell line was validated using a luciferase reporter cell-based assay. In this assay, Jurkat T cells expressing the luciferase reporter under the control of NFAT response elements are co-cultivated with TCR activator CHO cells. TCR complexes on Jurkat cells are activated by the TCR activator expressed in CHO cells, resulting in induction of the NFAT-dependent luciferase reporter.

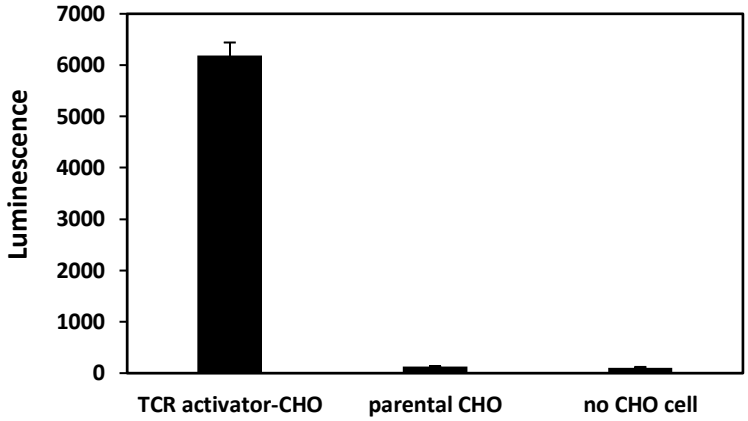


Figure 1: Co-culture of TCR activator CHO cells with NFAT Luciferase Reporter Jurkat cells.
TCR activator CHO cells and parental CHO cells were seeded in a white, clear-bottom 96-well plate. The following day, NFAT Luciferase Reporter Jurkat cells were added to the CHO cells. A negative control consisting of reporter cells without CHO cells was also performed. Induction of luciferase by the TCR activator was quantified using ONE-Step™ Luciferase System (BPS Bioscience 60690). Results are shown as raw luminescence signal.

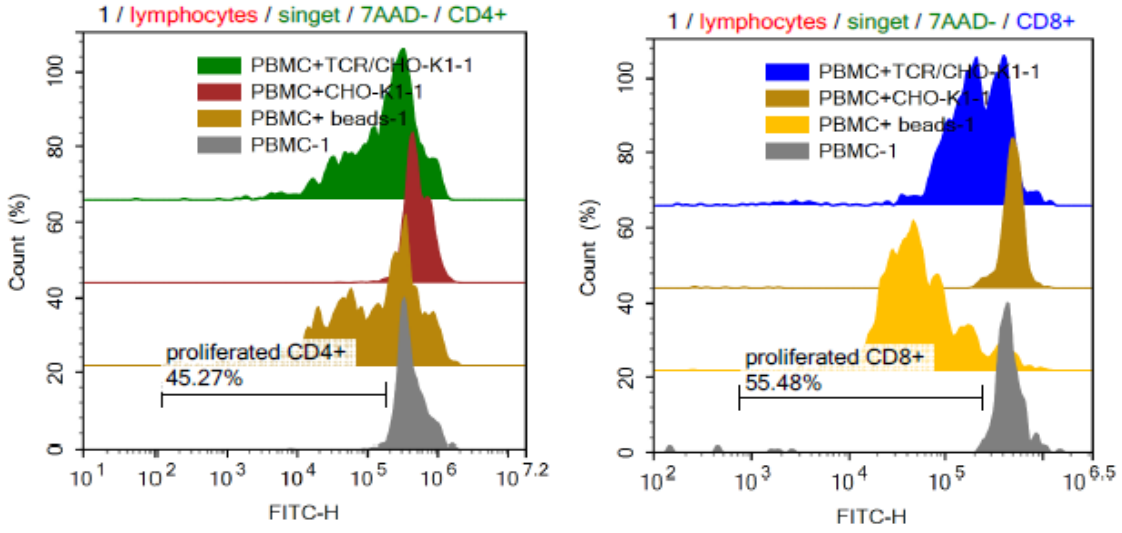


Figure 2: TCR activator CHO cells promote the proliferation of T cells.
Human Peripheral Blood Mononuclear Cells (PBMCs) were stained with CellTrace™ CFSE (ThermoFisher # C34554) and co-cultured with TCR-activator CHO, wildtype CHO (PBMC+CHO-K1), or were activated with anti-CD3/CD28 beads (PBMC + beads). Untreated (PBMC) were used as negative control. After 72 hours, cell proliferation was determined by flow cytometry analysis. Left: CD4+ proliferation; Right: CD8+ proliferation.

References

Courtney AH, Lo WL, Weiss A. TCR Signaling: Mechanisms of Initiation and Propagation. *Trends Biochem Sci.* (2018) **43(2)**: 108-123.

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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.

Related Products

Products	*Catalog #*	*Size*
TCR activator/PD-L1 CHO cell line	60536	2 vials
NFAT Reporter Jurkat cell line	60621	2 vials
TCR Knockout Jurkat Cell Line	78539	2 vials
TCR/B2M Knockout NFAT Luciferase Reporter Jurkat Cell Line	78364	2 vials
TCR Activator Lentivirus (CMV Promoter/Puromycin) or (EF1a Promoter/Puromycin) or (EF1a Promoter/Hygromycin)	79894	500 µl x 2