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Data Sheet

TCR Activator - CHO Recombinant Cell line
Cat#: 60539

Product Description

Recombinant CHO-K1 cell constitutively expressing a membrane bound, engineered T cell receptor (TCR) activator.

Application

- *in vitro* stimulation of T cells.
- Control cell line for TCR activator / PD-L1 - CHO cell line, BPS Cat. #60536

Format

Each vial contains 2.5 X 10^6 cells in 1 ml of 10% DMSO

Storage

Immediately upon receipt, store in liquid nitrogen.

Mycoplasma Testing

The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 3 (BPS Cat. #60186): Ham's F-12 medium (Hyclone # SH30526.01) supplemented with 10% FBS (Life technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone SV30010.01).

Growth Medium 3B (BPS Cat. #79529): Thaw Medium 3 plus 500 µg/ml of Hygromycin B (Hyclone #SV30070.01) to ensure the recombinant expression.

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3B. TCR activator - CHO cells should exhibit a typical cell division time of ~24 hours.

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, transfer to a tube containing 10 ml of Thaw Medium 3 **(no Hygromycin B)**, spin down the cells, and resuspend the cells in pre-warmed Thaw Medium 3 **(no Hygromycin B)**. Transfer resuspended cells to a T-25 flask and culture at 37°C in a CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells

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are ready to be split. Cells should be split before they reach complete confluence. At first passage, switch to Growth Medium 3B (**contains Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. Add Growth Medium 3B and transfer to a tube, and spin down the cells. Re-suspend the cells and seed appropriate aliquots of cell suspension into new culture vessels. Subcultivation ration: 1:10 to 1:20 twice a week.

Functional Validation and Assay Performance

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

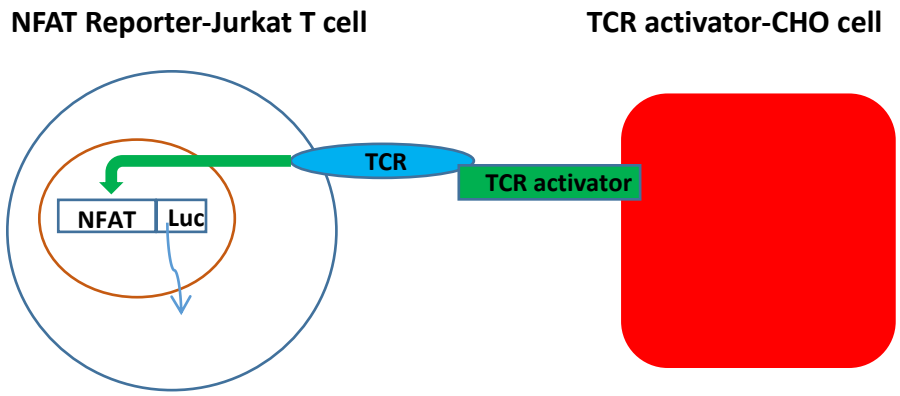

Figure 1. Co-culture of TCR activator-CHO cells with NFAT Reporter-Jurkat cells induced NFAT reporter expression in Jurkat cells.

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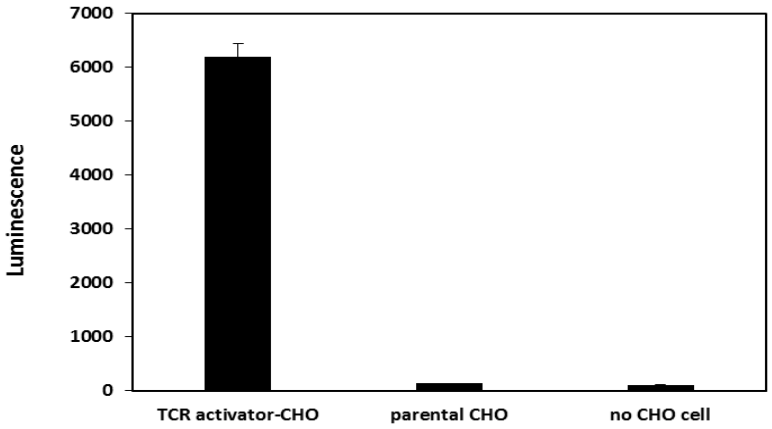
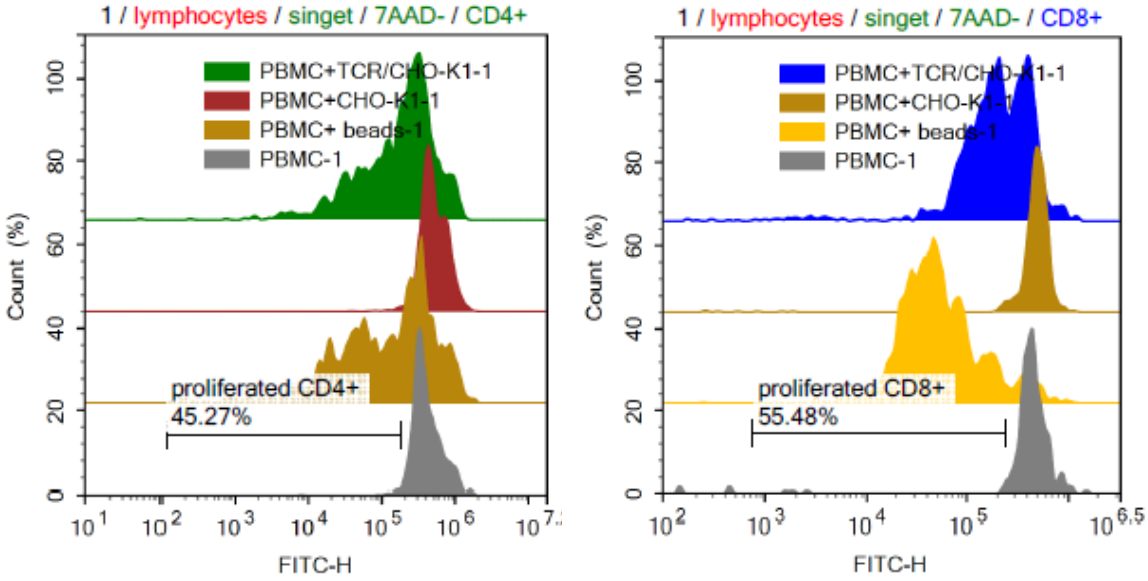


TCR activator-CHO cells or parental CHO cells were seeded in 96-well plate. The next day, NFAT Reporter-Jurkat cells (BPS Cat. #60621) were added to TCR activator-CHO cells. After incubation, ONE-Step™ Luciferase reagent (BPS Cat. #60690) was added to the cells to measure NFAT activity.

Figure 2. TCR activator/CHO-K1 promotes proliferation of T cells.



PBMCs were stained with CellTrace™ CFSE (Thermo Fisher Cat # C34554) and co-cultured with TCR-activator CHO-K1, wildtype CHO-K1 (PBMC+CHO-K1), activated with anti-CD3/CD28 beads (PBMC + beads), or untreated (PBMC) for 72 hours. (Left) CD4+ proliferation ; (Right) CD8+ proliferation.

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Related Products

Product	Cat. #
NFAT Reporter – Jurkat cell line	60621
PD-1/NFAT Reporter-Jurkat cell line	60535
TIGIT/NFAT Reporter – Jurkat cell line	60538
TCR activator / PD-L1 - CHO cell line	60536
TCR activator / PD-L1 expression kit	60610
TCR activator / PD-L2 expression kit	60620
ONE-Step™ Luciferase Assay System	60690

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