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Data Sheet

TCR activator/ HVEM - CHO Recombinant Cell Line
Catalog #: 79551

Product Description
Recombinant CHO-K1 cells constitutively expressing human HVEM (Herpes Virus Entry Mediator), also known as CD270 or TNFRSF14 (Tumor Necrosis Factor Receptor Superfamily Member 14), GenBank Accession No. NM_003820, and an engineered T cell receptor (TCR) activator.

Background
HVEM is a member of the TNFR superfamily that is expressed on T cells, B cells, NK cells and antigen-presenting cells. HVEM is a bidirectional switch that regulates T-cell activation in either a costimulatory or coinhibitory fashion, depending on the binding partner. HVEM can act as both ligand and receptor; the binding of HVEM to BTLA (IgSF) or CD160 on Effector T cells delivers a coinhibitory signal; alternately, the binding of HVEM to either of two tumor necrosis factor ligands, LIGHT or lymphotoxin-α, delivers a costimulatory signal. The HVEM/BTLA axis are co-inhibitory immune checkpoint molecules that play important roles in the blockade of T cell-mediated immune responses. The binding of BTLA, a receptor expressed on activated T-cells, to its ligand HVEM, found on melanoma and other cancers, negatively regulates T cell immune responses and allows cancer cells to escape immune surveillance. This co-inhibitory signal of the HVEM/BTLA interaction can be reversed by treatment with a BTLA blocking antibody. The HVEM/BTLA/LIGHT pathway is also involved in regulating autoimmune responses, making these proteins promising therapeutic targets for a number of diseases.

Application
- Screen for activators or inhibitors of BTLA/HVEM interaction in cell-based co-inhibitory bioassay.
- Characterize the biological activity of HVEM and its interactions with its receptor BTLA.
- Characterize T cell-mediated immune responses through BTLA and its interactions with HVEM.
- Screen novel co-inhibitory immune checkpoint molecules for cancer immunotherapy

Host Cell
CHO-K1

Format
Each vial contains 2 x 10^6 cells in 1 ml of 10% DMSO

Storage
Immediately upon receipt, store in liquid nitrogen.

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Mycoplasma Testing
The cell line has been screened using the PCR-based Venor®GeM Mycoplasma Detection kit (Sigma-Aldrich, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 3 (BPS Bioscience #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 3A (BPS Bioscience #60188): Thaw Medium 3 (BPS Bioscience #60186) plus 1 mg/ml of Geneticin (Life Technologies #11811031) and 500 µg/ml of Hygromycin B (Hyclone #SV30070.01) to ensure recombinant expression.

Cells should be grown at 37°C with 5% CO_2 using Growth Medium 3A (BPS Bioscience #60188). TCR activator/ HVEM - 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 37C water-bath, transfer to a tube containing 10 ml of Thaw Medium 3 **(no Geneticin or Hygromycin B)**, spin down cells, and re-suspend cells in pre-warmed Thaw Medium 3 **(no Geneticin or Hygromycin B)**. Transfer re-suspended cells to a T25 flask and culture at 37°C in a 5% CO_2 incubator overnight. The next day, replace the medium with fresh Thaw Medium 3 **(no Geneticin or Hygromycin B)**, and continue growing culture in a CO_2 incubator at 37°C until the cells are ready to be split. Cells should be split before they reach complete confluence. At first passage switch to Growth Medium 3A **(contains Geneticin and Hygromycin B)**.

To passage the cells, rinse cells with phosphate buffered saline (PBS), detach cells from culture vessel with 0.05% Trypsin/EDTA, and add Growth Medium 3A **(contains Geneticin and Hygromycin B)**. Transfer to a tube, spin down cells, re-suspend cells and seed appropriate aliquots of cell suspension into new culture vessels. Sub cultivation ration: 1:10 to 1:20 twice a week.

To freeze down the cells, rinse cells with phosphate buffered saline (PBS), and detach cells from culture vessel with 0.05% Trypsin/EDTA. After detachment, add Thaw Medium 3 **(no Geneticin or Hygromycin B)** and count the cells, then transfer to a tube, spin down cells, and resuspend in 4°C Freezing Medium (10% DMSO + 90% FBS) at ~2 x 10^6 cells/ml. Dispense 1 ml of cell aliquots into cryogenic vials. Place vials in an insulated container for slow cooling and store at -80°C overnight. Transfer to liquid nitrogen the next day for storage. It is recommended to expand the cells and freeze down more than 10 vials of cells at an early passage for future use.

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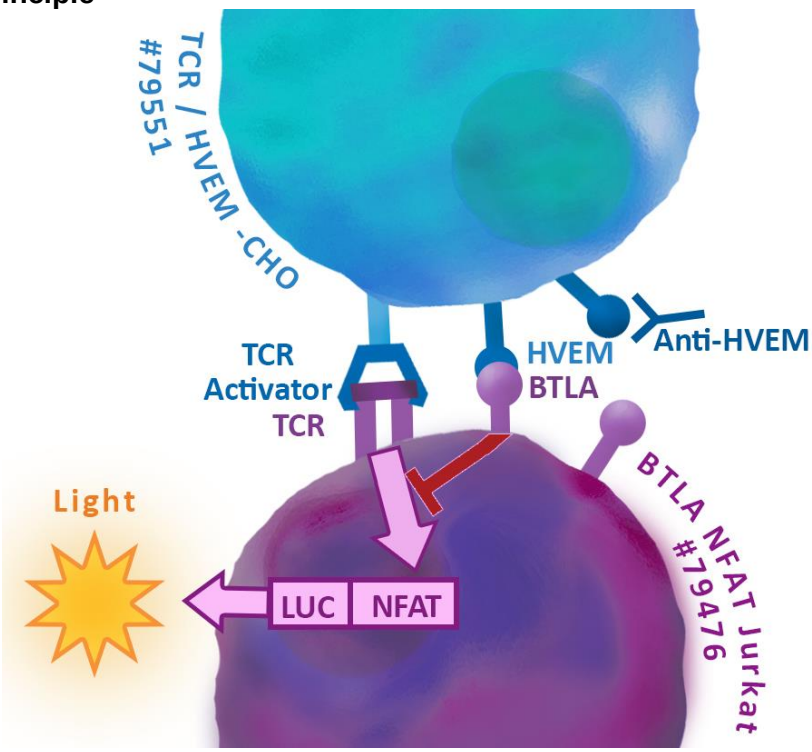
Assay Principle



Functional Validation and Assay Performance

Expression of Human HVEM in CHO-K1 cell line stably expressing TCR activator was confirmed by FACS using PE-anti-HVEM mAb.

The functionality of the cell line was validated using a BTLA:HVEM co-culture cell-based assay. In this assay, Jurkat T cells expressing NFAT reporter with constitutive expression of BTLA (BTLA/NFAT Reporter/Jurkat, BPS Bioscience #79476) are used as effector cells; TCR activator/ HVEM-CHO cells are used as target cells. When these two cells are co-cultivated, TCR complexes on effector cells are activated by TCR activator on target cells, resulting in expression of the NFAT luciferase reporter. However, BTLA and HVEM ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity. The inhibition of NFAT-Luciferase activity mediated by BTLA:HVEM interaction can be specifically reversed by anti-BTLA or anti-HVEM neutralizing antibodies (N/A). BTLA/HVEM neutralizing antibodies block BTLA:HVEM interaction and promote T cell activation, resulting in re-activation of the NFAT responsive luciferase reporter.

Materials Required but Not Supplied

- BTLA/Jurkat_NFAT reporter cell line (BPS Bioscience #79476)
- Assay medium: Thaw Medium 2 (BPS Bioscience #60184)

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- Growth Medium 3A (BPS Bioscience #60188)
- Thaw Medium 3 (BPS Bioscience #60186) Anti-BTLA neutralizing antibody (for testing, n/a)
- Anti-HVEM neutralizing antibody (for testing, n/a)
- 96-well tissue culture-treated white wall clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience #60690)
- Luminometer

Asaay protocol:

1. Harvest TCR activator/HVEM-CHO cells from culture and seed cells at a density of 10,000-35,000 cells per well into white clear-bottom 96-well microplate in 100 µl of Thaw Medium 3, without Geneticin or Hygromycin B. *Note: it is important to optimize the CHO cell number seeded per well; the optimal number of cells can vary, based on the passage number and culture density prior to seeding*. Incubate cells at 37°C in a CO_2 incubator for overnight. Cells should reach ~80% confluency on the next day (cells should not reach confluency in this step).
2. Next day, prepare serial dilution of anti-BTLA antibody or anti-HVEM antibody in Thaw Medium 2 (assay medium for Jurkat cells) The concentration of antibody here should be 2x the final treatment concentration of antibody). Harvest the BTLA/NFAT-reporter-Jurkat cells (log phase) by centrifugation and resuspend in assay medium. Dilute cells to 4×10^5 / ml in assay medium.
3. **To test anti-BTLA antibody,** preincubate the BTLA/NFAT Reporter- Jurkat cells (4×10^5 / ml) with diluted anti-BTLA antibody (1:1 in volume) for 30 min. After incubation, remove the medium from TCR activator/HVEM-CHO cells and add 100 µl of BTLA/NFAT reporter – Jurkat cells / anti-BTLA antibody mixture to the wells. (Note: Mix the BTLA/NFAT Reporter- Jurkat cells with antibody thoroughly before adding to TCR activator/HVEM-CHO cells.)
4. **To test anti-HVEM antibody,** remove the medium from TCR activator/HVEM-CHO cells, add 50 µl of diluted anti-HVEM antibody to the wells and incubate for 30 min. After incubation, add 50 µl of BTLAS/NFAT Reporter- Jurkat cells (4×10^5 / ml) to the wells. (Note: Mix the BTLA/NFAT Reporter- Jurkat cells well before adding to TCR activator/HVEM-CHO cells.)
5. The final cell density of the BTLA/NFAT Reporter- Jurkat cells is 2×10^4 /well. Set up each treatment in at least triplicate.
6. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).
7. Incubate the plates at 37°C in a CO_2 incubator for 5 to 6 hours.
8. After ~5 to 6-hour incubation, perform luciferase assay using the ONE-Step luciferase assay system, according to the recommended protocol. Add 100 µl of ONE-Step

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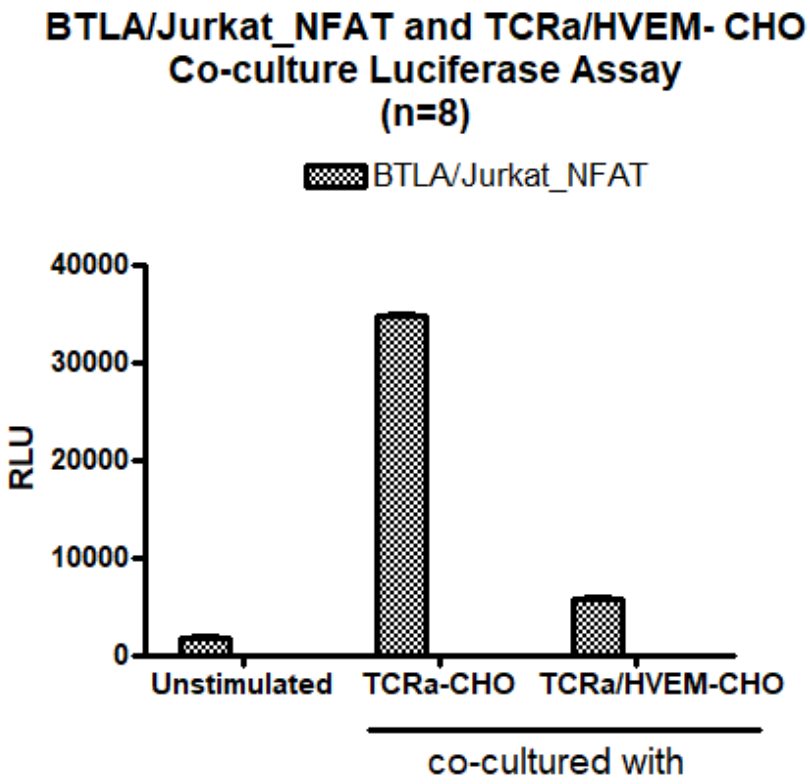
Luciferase reagent per well and rock gently at room temperature for ~30 minutes. Measure luminescence using a luminometer,.

9. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.

Data:

Figure 1. Co-culture of TCR activator/HVEM - CHO stable cell line with BTLA/Jurkat_NFAT reporter cells (BPS Bioscience 79476):



1A. TCR activator/HVEM-CHO cells and control TCR activator-CHO cells (no HVEM) were co-cultured with BTLA/NFAT Reporter-Jurkat cells: co-culturing with TCR activator CHO alone induced NFAT luciferase reporter activity by almost 20-fold through TCR activation, while co-culturing with TCR activator/HVEM-CHO cells reduced the luciferase reporter activity by 5 fold, indicating the inhibition of BTLA-mediated NFAT-luciferase activity in BTLA/NFAT Reporter-Jurkat cells is HVEM dependent.

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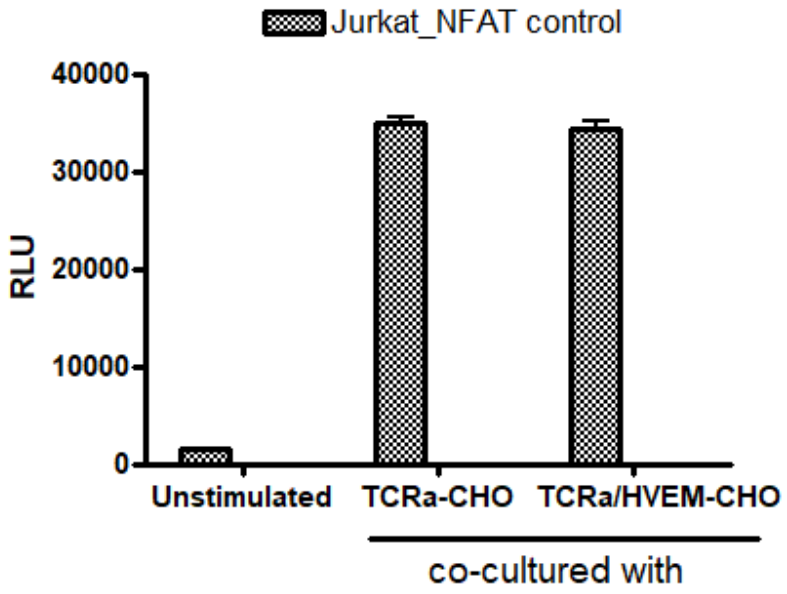
Control Jurkat_NFAT Reporter Cells and TCRa/HVEM-CHO Co-culture Luciferase Assay (n=8)



1B. TCR activator/HVEM-CHO cells and TCR activator-CHO cells (no HVEM) were co-cultured with control NFAT Reporter-Jurkat cells (no BTLA). Neither TCR activator/HVEM-CHO nor TCR activator-CHO cells showed any inhibition of luciferase activity in control Jurkat_NFAT Reporter cells (without BTLA) activated by TCRa. This data, in combination with figure 1A, indicating that the HVEM inhibitory effect on TCR activator-mediated luciferase activity is dependent on HVEM:BTLA interaction.

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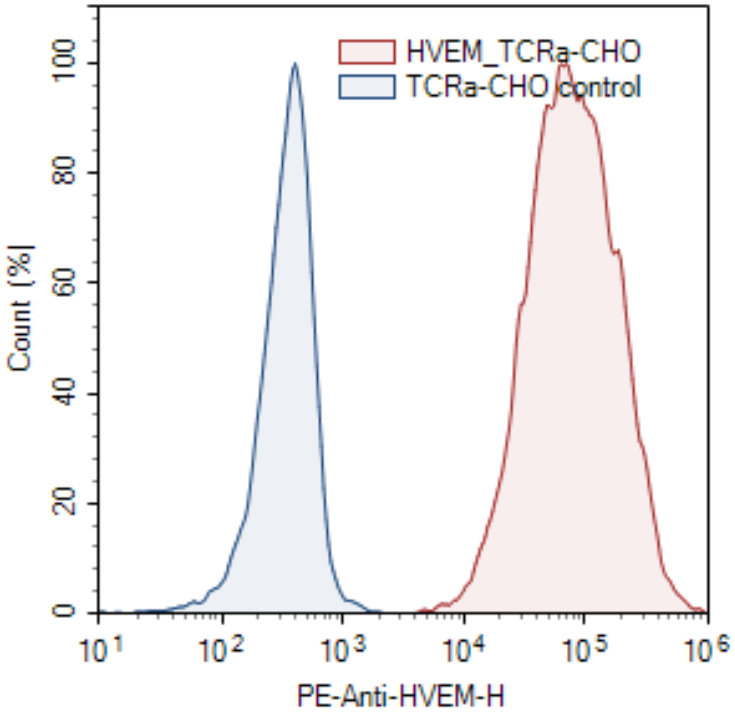
Figure 2. FACS analysis of cell surface expression of HVEM in TCR activator/HVEM-CHO stable cells: TCRa/HVEM-CHO and TCRa-CHO control cells were stained with PE-labeled anti-HVEM antibody (BioLegend, #318806) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.



Vector and Sequence
Human HVEM (NM_003820.2) was cloned into pIRESNeo.

Sequence:
MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKCSPGYR
VKEACGELTGTVCEPCPPGTYIAHLNGLSKCLQCQMCDPAMGLRASRNCSRTENAVCGCSPG
HFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPPGTFSPNGTLEECQHQTKC
SWLVTKAGAGTSSSHWVWWFLSGSLVIVIVCSTVGLIICVKRRKPRGDVVKVIVSVQRKRQEAE
GEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH

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Related Products

Product	Cat. #	Size
BTLA/NFAT-Luc Reporter-Jurkat Cell Line	79476	2 vials
TCR Activator - CHO Recombinant Cell line	60539	2 vials
TCR Activator/HVEM Mammalian Expression Kit	79489	500 rxns
NFAT Reporter – Jurkat cell line	60621	2 vials
ONE-Step™ Luciferase Assay System	60690-1	10 ml
ONE-Step™ Luciferase Assay System	60690-2	100 ml
BTLA:HVEM[Biotinylated] Inhibitor Screening Assay Kit	72008	96 rxns
BTLA(CD272), Fc fusion (Human)	71141	100 µg
HVEM, Fc fusion (Human)	71142	100 µg
HVEM/NF-κB Reporter Jurkat Recombinant Cell Line	79310	2 vials
HVEM - CHO Recombinant Cell Line	79297	2 vials
HVEM - HEK293 Recombinant Cell Line	79313	2 vials
LIGHT-CHO Recombinant Cell Line	79262	2 vials

Notes

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