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Data Sheet

BTLA / NFAT - Luciferase Reporter - Jurkat Recombinant Cell Line

Catalog #: 79476

Product Description

Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of human BTLA (B and T lymphocyte associated, B and T lymphocyte attenuator, BTLA1, CD272, GenBank Accession #NM_181780).

Background

BTLA is an immunoglobulin domain-containing glycoprotein expressed on T cells, resting B cells, macrophages, DCs and, to a lesser extent, NK cells. BTLA acts as an inhibitory receptor on T cells, as anti-BTLA treatment results in T cell proliferation, and BTLA knockout mice demonstrate hyper-responsive immune activation. Subsequently, herpesvirus entry mediator (HVEM), a tumor necrosis factor receptor, was identified as a natural ligand for BTLA in mice and humans. Expression of HVEM by antigen-presenting cells (APCs) was capable of inducing BTLA-dependent T cell inhibition.

BTLA belongs to the immunoglobulin superfamily, along with CTLA-4 and PD-1, which characteristically binds to B7 family members. HVEM is a member of the tumor necrosis factor receptor family, and the BTLA/HVEM interaction is the first to demonstrate crosstalk between these two receptor families. Prior to the discovery of the BTLA/HVEM interaction, HVEM was known to bind LIGHT and lymphotoxin-α, both tumor necrosis factor ligands. While the BTLA/HVEM interaction results in a co-inhibitory signal, the HVEM/LIGHT interaction results in a co-stimulatory signal through HVEM.

In melanoma patients, BTLA was demonstrated to be expressed on tumor-specific T cells both in circulation and in metastatic lymph nodes. HVEM was subsequently shown to be expressed in the majority of cultured melanoma cell lines and metastatic melanoma samples. The interaction of BTLA on tumor specific T cells and HVEM on melanoma cells resulted in T cell inhibition, which could be reversed by treatment with a BTLA blocking antibody. BTLA/HVEM interaction plays a key role in the regulation of inflammatory, autoimmune, and antitumor responses, and is an important target for cancer immunotherapy drug discovery. Therapeutically targeting BTLA and HVEM remains in pre-clinical stages as the bidirectional signaling pathways of BTLA/HVEM and HVEM/LIGHT are further elucidated.

Application

- Screen for activators or inhibitors of BTLA/HVEM interaction in cell-based co-inhibitory bioassay.
- Characterize the biological activity of BTLA and its interactions with ligands.
- Characterize T cell-mediated immune responses through BTLA and its interactions with HVEM.

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- Screen novel co-inhibitory immune checkpoint molecules for cancer immunotherapy

Host Cell
Jurkat T cell

Format
Each vial contains 2 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, #MP0025) to confirm the absence of *Mycoplasma* species.

General Culture Conditions

Thaw Medium 2 (BPS Cat. #60184): RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01),

Complete Growth Medium: Thaw Medium 2 plus 1 mg/ml of Geneticin (Life Technologies #11811031) and 200 µg/ml of Hygromycin B (Hyclone #SV30070.01).

Cells should be grown at 37°C with 5% CO_2 using complete growth medium (Thaw Medium 2 plus Geneticin and Hygromycin B)

It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37°C water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 **(no Geneticin and Hygromycin B)**. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator. After 24 hours of culture, add an additional 3 – 4 ml of Thaw Medium 2 **(no Geneticin and Hygromycin B)**. At first passage, switch to complete growth medium **(contains Geneticin and Hygromycin B)**. Cells should be split before they reach 2 x 10^6 cells/ml. Note: This cell line tends to grow more slowly than parental Jurkat cells.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.2 x 10^6 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Functional Validation and Assay Performance
Expression of Human BTLA in Jurkat cell line was confirmed by FACS using PE-anti-BTLA mAb.

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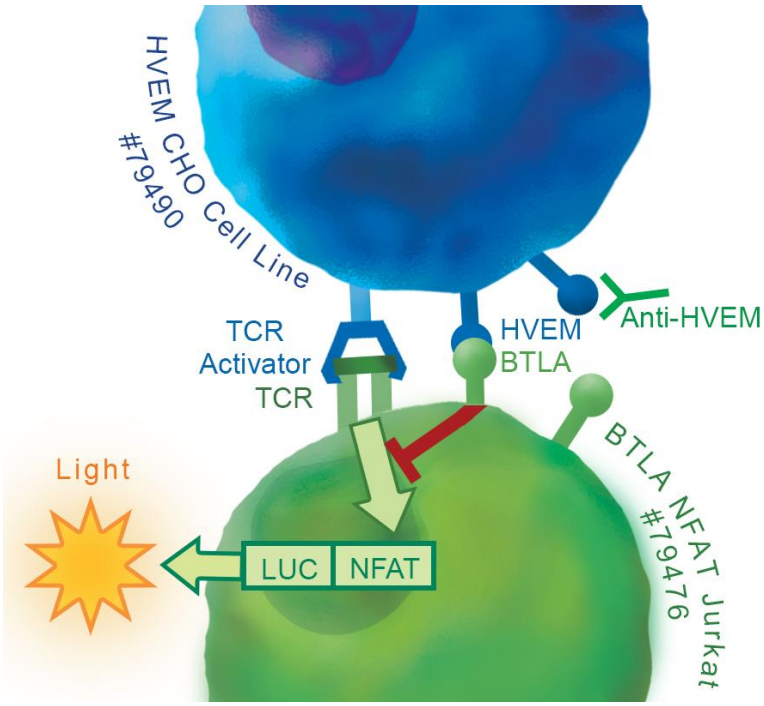
The functionality of the cell line was validated using a HVEM:BTLA co-culture bioassay. In this assay, BTLA/NFAT Reporter/Jurkat stable cells are used as effector cells; CHO cells over-expressing HVEM and an engineered T cell receptor (TCR) activator are used as target cells. When these two cells are 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; when the BTLA/NFAT Jurkat reporter cells were co-cultured with a negative control target cell PD-L1/CHO_TCR activator, no inhibition is observed. This inhibition can be specifically reversed by anti-BTLA or anti-HVEM blocking antibodies. BTLA/HVEM neutralizing antibodies block BTLA:HVEM interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter (data not shown).



Materials Required but Not Supplied

- CHO-K1 cell and its growth medium
- TCR Activator/HVEM Mammalian Expression Kit (BPS Bioscience, coming soon), including TCRa/pIRES expression vector and HVEM/pIRES expression vector.
- HVEM/CHO_TCR activator stable cell line (BPS Bioscience, coming soon)
- Transfection reagent for generating target cells (aka artificial Antigen Presenting Cells (aAPC)) [We use Lipofectamine™ 2000 (Life technologies #11668027). However, other transfection reagents work equally well.]
- Opti-MEM I Reduced Serum Medium (Life technologies #31985-062)
- Assay medium: Thaw Medium 2 (BPS Cat. # 60184)
- Anti-BTLA neutralizing antibody (for testing)

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- Anti-HVEM neutralizing antibody (for testing)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS Bioscience # 60690)
- Luminometer

Asaay protocol:

1. One day before transfection, seed CHO-K1 cells in 96 well plate at a density of 35,000 cells per well in 100 µl of growth medium so that cells will be 90% confluent at the time of transfection.

2. Next day, transfect CHO-K1 cells with expression vectors for engineered TCR activator and human HVEM (or PD-L1 as control) following the manufacturer's protocol; we used 0.2 ng TCRa/pIRES and 50 ng HVEM/pIRES or PD-L1/pIRES for each well.

3. One day after transfection, prepare serial dilution of antibody in assay medium (the concentration of antibody here is 2x of the final treatment concentration of antibody). Harvest the BTLA/NFAT-reporter-Jurkat cells by centrifugation and resuspend in assay medium. Dilute cells to 4 x 10^5 / ml in assay medium.

   To test anti-BTLA blocking antibody, preincubate the BTLA/NFAT Reporter- Jurkat cells (4 x 10^5 / ml) with diluted antibody (1:1 in volume) for 15-30 min. After incubation, remove the medium from transfected CHO-K1 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 immediately before adding to transfected CHO-K1 cells.)*

   To test the anti-HVEM antibody, remove the medium from transfected CHO-K1 cells, add 50 µl of diluted anti-HVEM antibody to the wells and incubate for 30 min. After incubation, add 50 µl of BTLA/NFAT Reporter- Jurkat cells (4 x 10^5 / ml) to the wells (Note: *Mix the PD-1/NFAT Reporter- Jurkat cells thoroughly immediately before adding to transfected CHO-K1 cells.)*

   Final cell density of BTLA/NFAT Reporter- Jurkat cells is 2 x 10^4 /well. Set up each treatment in at least triplicate.

   Add 100 µl of assay medium to cell-free control wells (for determining background luminescence).

4. After 6~16 hours, measure the luciferase expression using the ONE-Step luciferase assay system, following the recommended protocol. Add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer.

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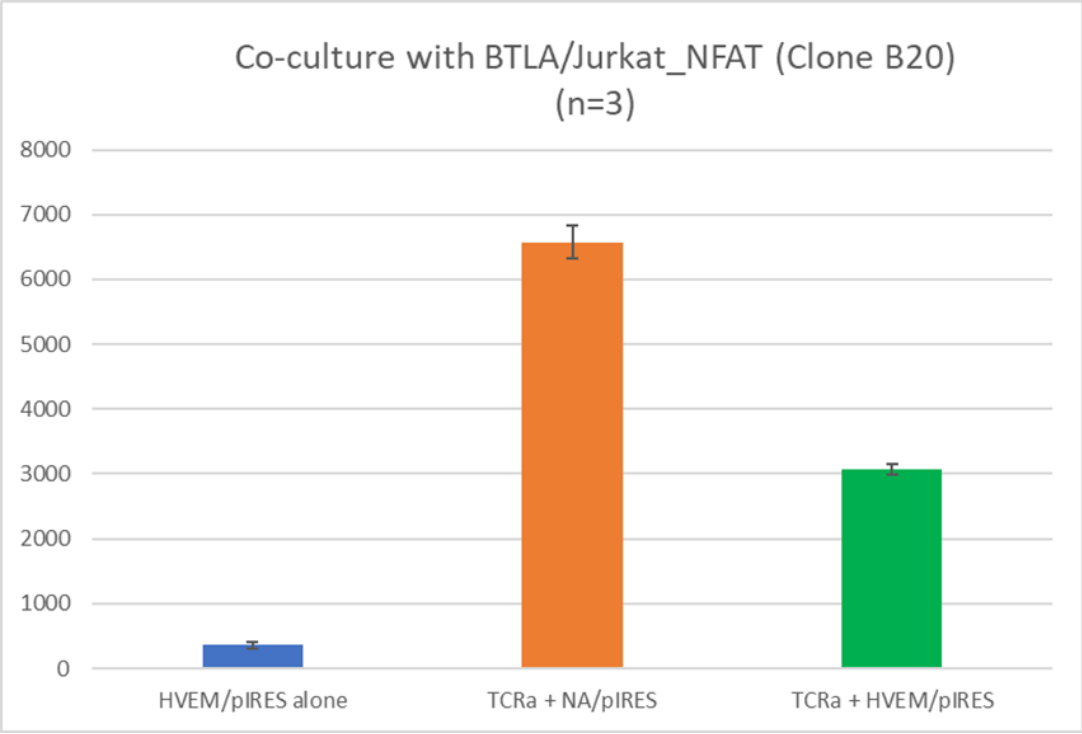
5. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells.
   The fold induction of NFAT luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Data:

Figure 1. Co-culture of BTLA/Jurkat_NFAT reporter cells (Clone B20) with CHO/TCR activator and CHO/TCR activator + HVEM.
CHO-K1 cells were transiently transfected with the genes for an engineered T cell receptor (TCR) activator, with or without human HVEM. The next day, BTLA/NFAT Reporter-Jurkat cells (or control NFAT Reporter – Jurkat cells) were co-cultured with transfected CHO-K1 cells. After ~16 hours of stimulation, ONE-Step™ Luciferase reagent (BPS Bioscience #60690) was added to the cells to measure NFAT activity.

A. After co-culture with BTLA/NFAT Reporter-Jurkat cells, CHO-K1 transfected with TCR activator and a control vector (NA; we usedPD-L1/pIRES) induced NFAT luciferase reporter activity by more than 20-fold through TCR activation (orange bar); while CHO-K1 transfected with TCR activator and HVEM/pIRES reduced the luciferase reporter activity from 20-fold to 10-fold (green bar)



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B. After co-culture with control cell line (NFAT Reporter - Jurkat cells), CHO-K1 transfected with HVEM and TCR activator didn't show any inhibition of luciferase activity at all (green bar), indicating that HVEM inhibitory effect on TCR activator-mediated luciferase activity is BTLA dependent.

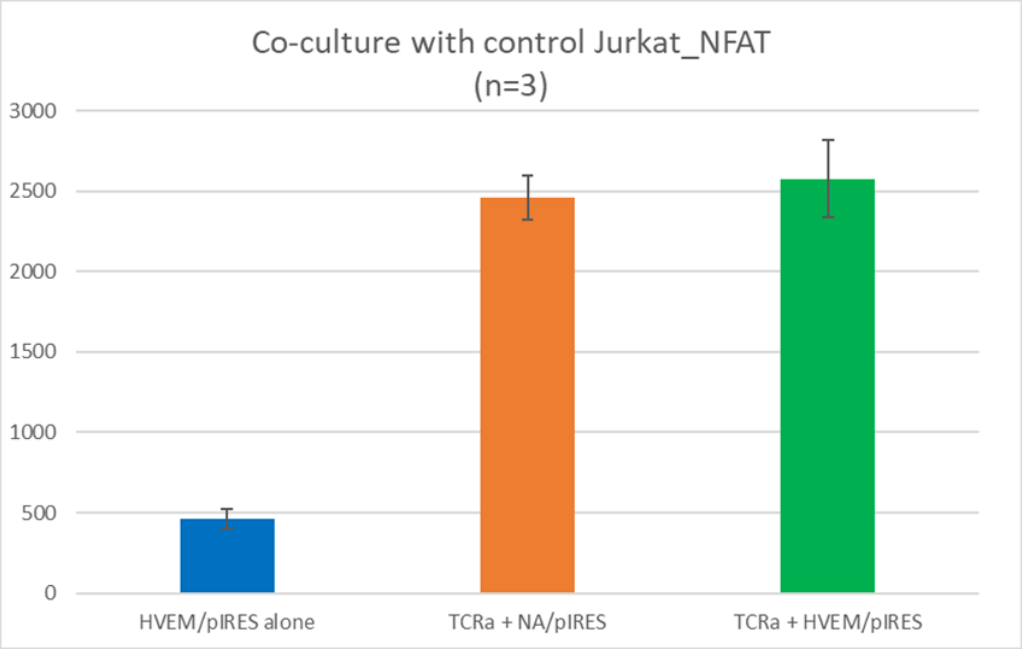

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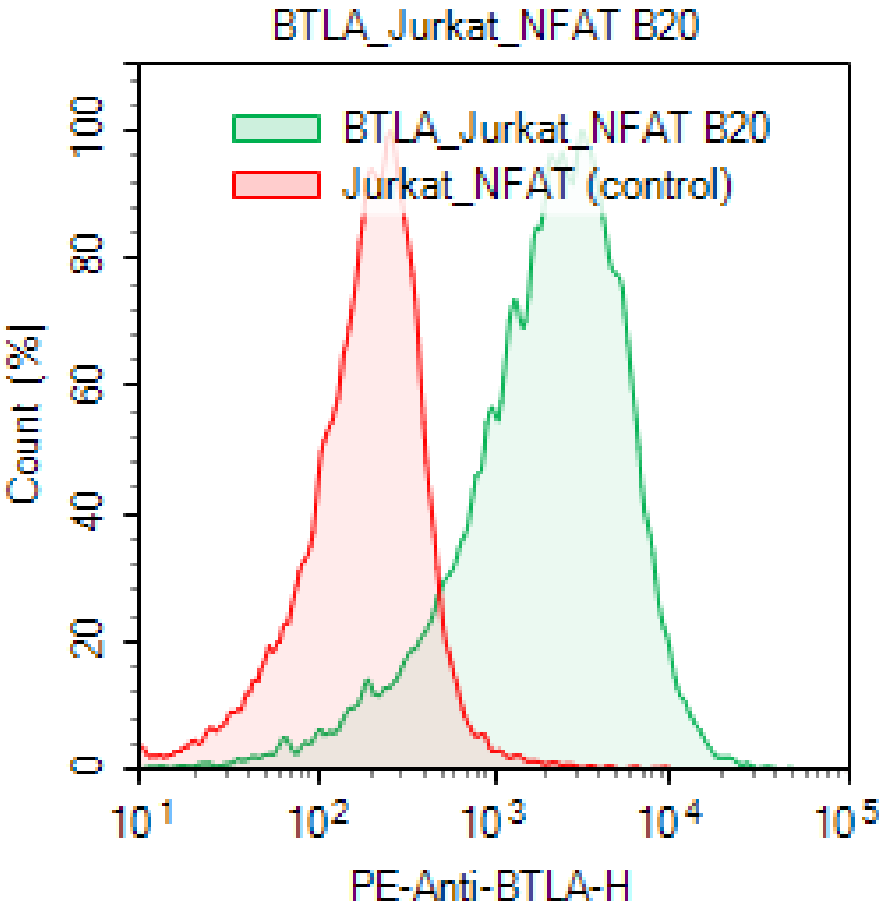
Figure 2. FACS analysis of cell surface expression of BTLA in BTLA/NFAT Reporter-Jurkat cells:
BTLA/NFAT Reporter-Jurkat cells or control NFAT Reporter-Jurkat cells were stained with PE-labeled anti-BTLA antibody (Biolegend 344506) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.



Sequence

BTLA sequence (accession number NM_181780):

MKTLPAMLGTGKLFWVFFLIPYLDIWNIHGKESCDVQLYIKRQSEHSILAGDPFELECPVKYCAN
RPHVTWCKLNGTTCVKLEDRQTSWKEEKNISFFILHFEPVLPNDNGSYRCSANFQSNLIESHST
TLYVTDVKSASERPSKDEMASRPWLLYSLLPLGGLPLLITTCFCLFCCLRRHQGKQNELSDTAG
REINLVDAHLKSEQTEASTRQNSQVLLSETGIYDNDPDLCFRMQEGSEVYSNPCLEENKPGIVY
ASLNHSVIGPNSRLARNVKEAPTEYASICVRS

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Related Products

Product	Cat. #	Size
TCR Activator/HVEM Mammalian Expression Kit	79489	500 rxns
HVEM/CHO_TCR activator stable cell line	79490	2 vials
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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