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# Data Sheet

## CD160/NFAT - Luciferase Reporter - Jurkat Recombinant Cell Line

## Catalog #: 79594

**PRODUCT DESCRIPTION:** Recombinant Jurkat T cell expressing firefly luciferase gene under the control of NFAT response elements with constitutive expression of human CD160. CD160 is a GPI-anchored glycoprotein member of the Ig superfamily, also known as BY55, NK1, and NK28. GenBank Accession # NM_007053.

**BACKGROUND:** CD160 is a glycosylphosphatidylinositol (GPI)-anchored protein member of the Ig superfamily that is expressed at the cell surface and highly restricted to circulating NK and T cells. Binding of CD160 to both classical and non-classical MHC I enhances NK and CD8+ CTL functions. However, engagement of CD160 by the Herpes Virus Entry Mediator (HVEM / TNFRSF14) was shown to mediate inhibition of CD4+ T-cell proliferation and TCR-mediated signaling. HVEM protein is a bimolecular switch that binds both co-stimulatory LT-α/LIGHT and co-inhibitory receptors CD160/BTLA. The binding of coinhibitory receptors CD160 and/or BTLA on T cells with HVEM expressed on DC or Tregs transduces negative signals into T cells that are counterbalanced by costimulatory signals delivered after direct engagement of HVEM on T cells by LIGHT expressed on DC or more likely, on other activated T cells (T–T cell cooperation). HVEM was also shown to be expressed in the majority of cultured melanoma cell lines and metastatic melanoma samples.

The predominance of the interaction of HVEM with CD160 and BTLA over the HVEM/LIGHT pathway or vice versa might be the result of differences in ligand/receptor affinity and the differential expression pattern of these molecules on cell types at different stages of cell differentiation. LIGHT, BTLA, and CD160 have substantially different binding affinities and occupy spatially distinct sites upon interaction with the HVEM receptor, which enables HVEM to function as a molecular switch. The net effect of the LIGHT/HVEM and HVEM/BTLA/CD160 interaction, when these different receptors and ligands are simultaneously present, determines the outcome of the response.

CD160/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. The interaction of CD160 on tumor specific T cells and HVEM on melanoma cells resulted in T cell inhibition, which could be reversed by treatment with anti-CD160 blocking antibody. Therapeutically targeting CD160 and HVEM remains a focus for in pre-clinical studies as the bidirectional signaling pathways of CD160/BTLA/HVEM and HVEM/LIGHT are further elucidated.

**APPLICATION:**

- Screen for activators or inhibitors of CD160/HVEM interaction in cell-based co-inhibitory bioassay.
- Characterize the biological activity of CD160 and its interactions with ligands.
- Characterize T cell-mediated immune responses through CD160 and its interactions with HVEM.
- Screen novel co-inhibitory immune checkpoint antibodies and molecules for cancer immunotherapy

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**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 Bioscience #60184):** RPMI1640 medium (Life Technologies #A10491-01) supplemented with 10% FBS (Life Technologies #26140-079), 1% Penicillin/Streptomycin (Hyclone #SV30010.01),

**Growth Medium 2A (BPS Bioscience #60190):** 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 2A (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 Growth Medium 2A **(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 CD160 in Jurkat_NFAT luciferase reporter cell line was confirmed by FACS using PE-anti-CD160 mAb.

The functionality of the cell line was validated using a HVEM:CD160 co-culture bioassay. In this assay, CD160/NFAT luciferase reporter/Jurkat stable cells are used as effector cells; CHO cells transiently 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, CD160 and HVEM ligation prevents TCR activation and suppresses the NFAT-responsive luciferase activity; when the CD160/NFAT Jurkat reporter cells were co-cultured with a negative control target cell PD-L1/CHO_TCR activator, no inhibition is observed.

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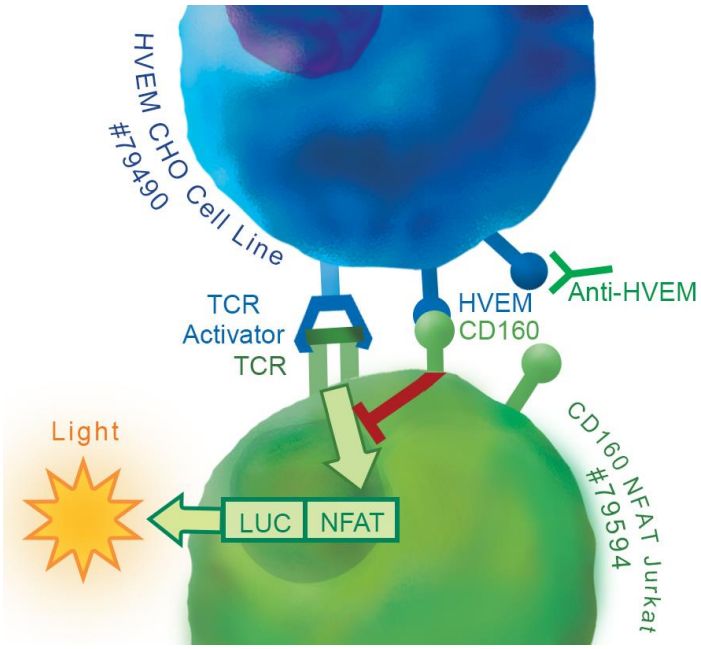
This inhibition can be specifically reversed by anti-CD160 or anti-HVEM blocking antibodies. CD160/HVEM neutralizing antibodies block CD160: HVEM interaction and promote T cell activation, resulting in reactivation of the NFAT responsive luciferase reporter (data not shown).




**MATERIALS REQUIRED BUT NOT SUPPLIED:**

- CHO-K1 cell and its growth medium
- TCR Activator/HVEM Mammalian Expression Kit (BPS Bioscience #79489), including TCRa/pIRES expression vector and HVEM/pIRES expression vector.
- HVEM/CHO_TCR activator stable cell line (BPS Bioscience #79551)
- 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 Bioscience #60184)
- Anti-BTLA neutralizing antibody (for testing)
- 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

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## ASSAY 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 TCRa/pIRES DNA titration from 0.2ng/well, plus 50 ng HVEM/pIRES or negative control plasmid 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-CD160 blocking antibody,** preincubate the CD160/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 CD160/NFAT reporter-Jurkat cells/anti-CD160 antibody mixture to the wells. (Note: *Mix the CD160/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 CD160/NFAT Reporter- Jurkat cells (4 x $10^5$ / ml) to the wells (Note: *Mix the CD160/NFAT Reporter- Jurkat cells thoroughly immediately before adding to transfected CHO-K1 cells.)*

   Final cell density of CD160/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.

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.

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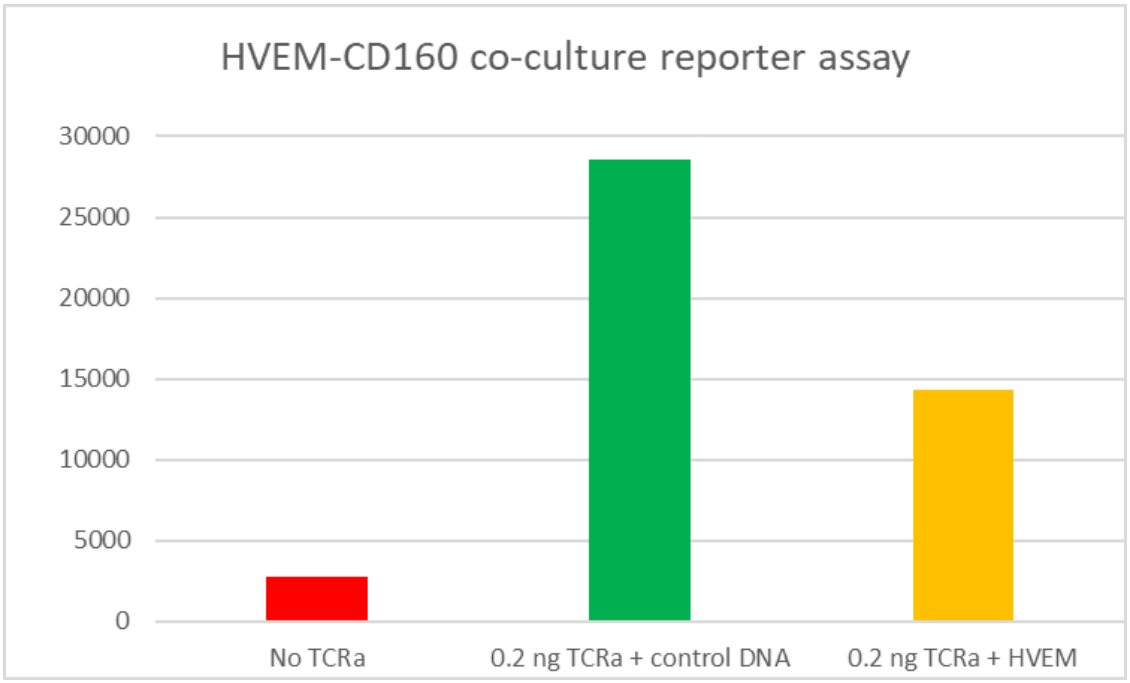
**DATA:**

**Figure 1. Co-culture of CD160/Jurkat_NFAT reporter cells 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, CD160/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 CD160/NFAT Reporter-Jurkat cells, CHO-K1 transfected with TCR activator and a control vector (PD-L1/pIRES) induced NFAT luciferase reporter activity by more than 10-fold through TCR activation (green bar); while CHO-K1 transfected with TCR activator and HVEM/pIRES reduced the luciferase reporter activity from 10-fold to 5-fold (orange 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 (blue bar), indicating that HVEM inhibitory effect on TCR activator-mediated luciferase activity is CD160 dependent.

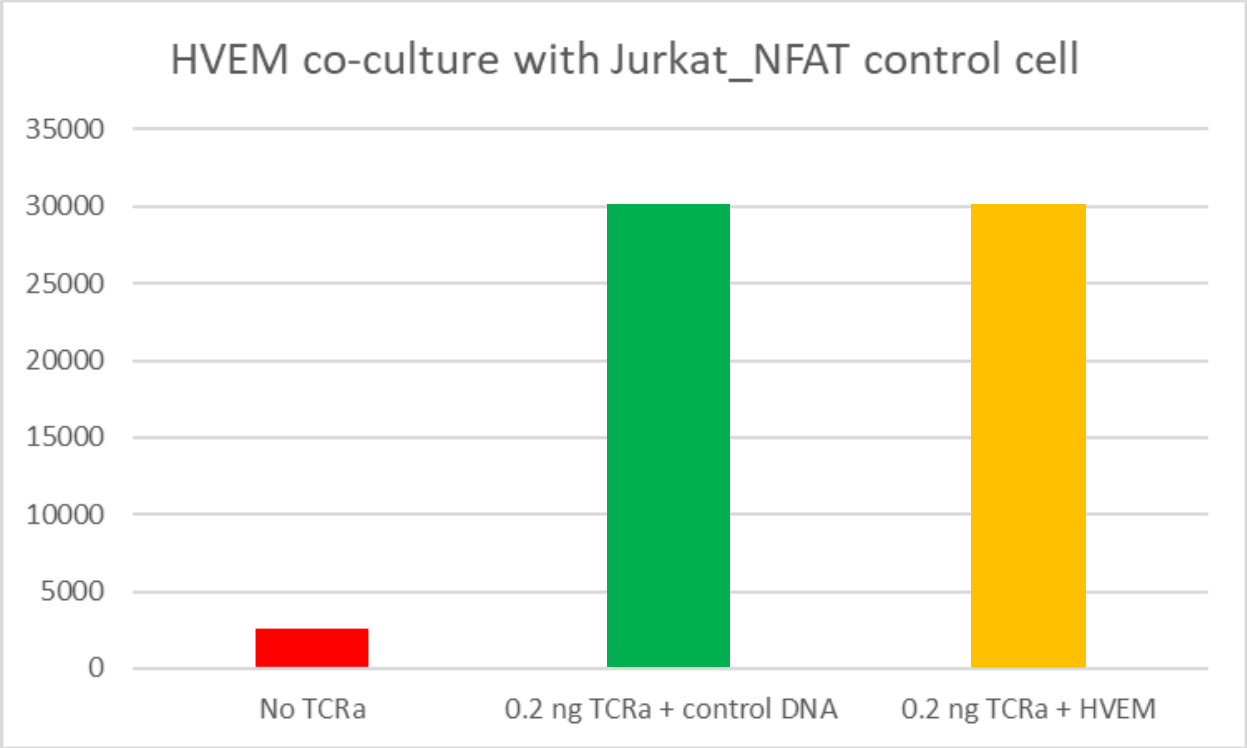

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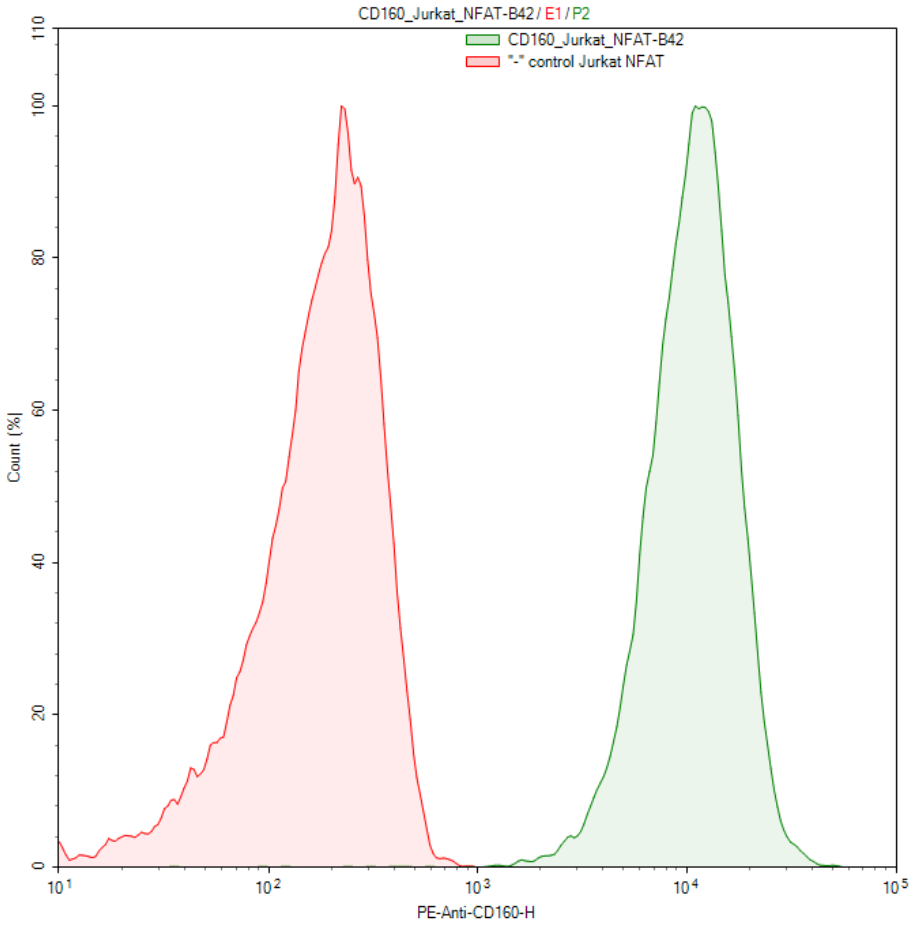
**Figure 2. FACS analysis of cell surface expression of CD160 in CD160/NFAT Reporter-Jurkat cells:**
BTLA/NFAT Reporter-Jurkat cells or control NFAT Reporter-Jurkat cells were stained with PE-labeled anti-CD160 antibody (Biolegend 341206) and analyzed by FACS. Y-axis is the cell count. X-axis is the intensity of PE.




**SEQUENCE:**

CD160 sequence (accession number NM_007053):

MLLEPGRGCCALAILLAIVDIQSGGCINITSSASQEGTRLNLICTVWHKKEEAEGFVVFLCKDRS
GDCSPETSLKQLRLKRDPGIDGVGEISSQLMFTISQVTPLHSGTYQCCARSQKSGIRLQGHFFS
ILFTETGNYTVTGLKQRQHLEFSHNEGTLSSGFLQEKVWVMLVTSLVALQAL

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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 | 79551 | 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 |
| BTLA / NFAT - Luciferase Reporter - Jurkat Cell Line | 79476 | 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 |

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