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Data Sheet

HVEM/NF-κB Reporter Jurkat Recombinant Cell Line
Catalog # 79310

Product Description
Recombinant clonal stable Jurkat T cell line expressing firefly luciferase gene under the control of 4 copies of NF-κB response elements with constitutive expression of human HVEM (Herpes Virus Entry Mediator, Tumor Necrosis Factor Receptor Superfamily Member 14, TNFRSF14, CD270, GenBank Accession No. NM_003820). Following activation by human HVEM ligand LIGHT, NF-κB transcription factors bind to the DNA response elements to induce transcription of the luciferase reporter gene.

Background
HVEM is a bidirectional switch regulating T-cell activation in a costimulatory or coinhibitory fashion whose outcome depends on the binding partner. HVEM can act as both receptor and ligand, the binding of endogenous ligand LIGHT or agonist antibodies to HVEM delivers a costimulatory signal; whereas the binding of HVEM to BTLA (IgSF) or CD160 on Effector T cells delivers a coinhibitory signal. LIGHT/HVEM axis are co-stimulatory immune checkpoint molecules extensively studied for cancer immunotherapy; LIGHT, either in the soluble monomer form or homotrimer expressed on the cell surface, can activate NF-κB through binding to HVEM; we have shown that LIGHT expressing cells are more potent and efficacious than the soluble LIGHT for activating HVEM expressed on T cell surface.

Application
- Screen for agonists or antagonists of LIGHT-HVEM signaling in a physiological relevant cellular context
- Characterize T cell-mediated immune responses of HVEM and its interactions with LIGHT
- Screen co-stimulatory 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 in FBS.

Storage
Store in liquid nitrogen immediately upon receipt.

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)

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Complete Growth Medium: Thaw Medium 2 (BPS Cat. #60184) plus 1 mg/ml G418 (Thermo Fisher, Cat. # 11811031) and 200 µg/ml of Hygromycin B (Hyclone #SV30070.01)

Cells should be grown at 37ºC with 5% CO2 using complete growth medium (Thaw Medium 2 with G418 and Hygromycin B).

Recommended Culture Condition
It is recommended to quickly thaw the frozen cells from liquid nitrogen in a 37ºC water-bath, then transfer the entire contents of the vial to a tube containing 10 ml of Thaw Medium 2 (no G418 and Hygromycin B). Spin down the cells, remove supernatant and resuspend cells in pre-warmed Thaw Medium 2 (no G418 and Hygromycin B). Transfer the resuspended cells to a T25 flask and incubate at 37ºC in a 5% CO2 incubator. This cell line tends to grow more slowly than parental WT Jurkat cells. After 24 hours of culture, add an additional 3 – 4 ml of growth medium without antibiotics. At first passage, switch to complete growth medium (contains G418 and Hygromycin B). Cells should be split before they reach $2x10^6$ cells/ml.

To passage the cells, dilute cell suspension into new culture vessels at no less than 0.1x106 cells/ml. Subcultivation ratio: 1:10 to 1:20 twice a week.

Mycoplasma Testing
This cell line has been screened using the MycoAlert™ Mycoplasma Detection Kit (Lonza, #LT07-118) to confirm the absence of Mycoplasma contamination. MycoAlert Assay Control Set (Lonza, #LT07-518) was used as a positive control.

Application References
1. Chen, *et.al.* (2013) Molecular mechanisms of T cell co-stimulation and co-inhibition. *Nat Rev Immunol. 2013 April; 13(4): 227–242*.
2. Steinberg, *et.al.* (2014) The Signaling Networks of the Herpesvirus Entry Mediator (TNFRSF14) in Immune Regulation. *Immunol Rev. 2011 November; 244(1): 169–187*.
3. Rio, *et.al.* (2014) Therapeutic blockade of LIGHT interaction with HVEM and LTβR attenuates in vivo cytotoxic allogeneic responses. *Transplantation. 2014 December 15; 98(11): 1165–1174.*
4. Shui *et.al.* (2014) HVEM is a TNF Receptor with Multiple Regulatory Roles in the Mucosal Immune System. *Immune Netw. 2014 Apr; 14(2): 67–72.*
5. Steinberg, *et.al.* (2009) Regulating the mucosal immune system: the contrasting roles of LIGHT, HVEM, and their various partners. *Semin. Immunopathol. 31: 207-221*.
6. Ware *et.al.* (2009) Targeting the LIGHT-HVEM pathway. *Adv.Exp.Med.Biol. 647:146*

Assay Principle

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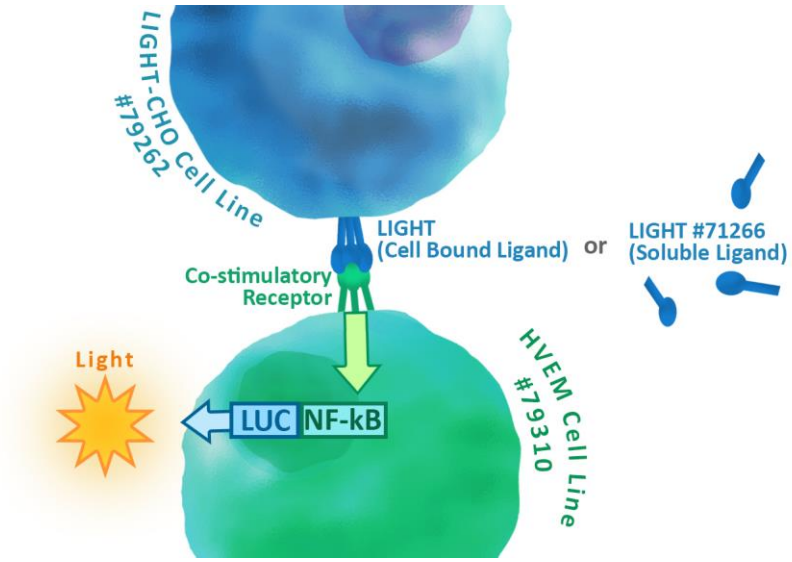

Quality Assurance

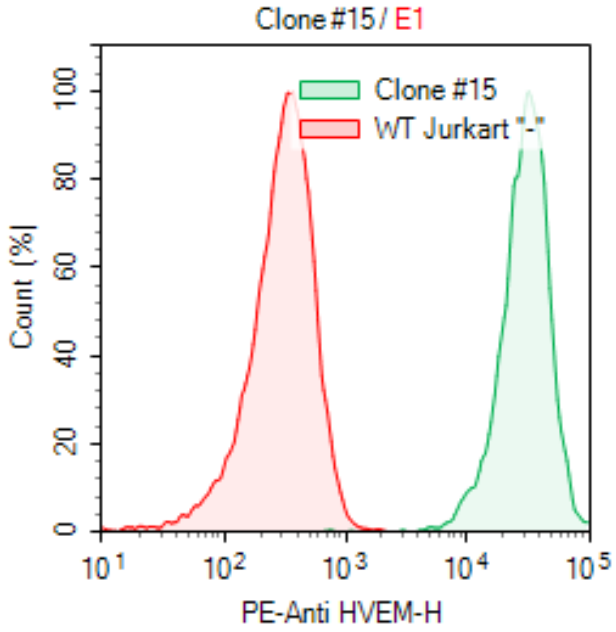

Figure 1. Expression of human HVEM validated by flow cytometry. Flow cytometry showed PE-conjugated anti-human HVEM antibody (BioLegend, #318806) detects HVEM-positive clonal population (clone B15) (green), using wild-type Jurkat cells as a negative control (red).

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

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a.a. sequence:

MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKCSPGYR
VKEACGELTGTVCEPCPPGTYIAHLNGLSKCLQCQMCDPAMGLRASRNCSRTENAVCGCSPG
HFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPPGTFSPNGTLEECQHQTKC
SWLVTKAGAGTSSSHWVWWFLSGSLVIVIVCSTVGLIICVKRRKPRGDVVKVIVSVQRKRQEAE
GEATVIEALQAPPDVTTVAVEETIPSFTGRSPNH

Materials Required but Not Supplied

- Assay Medium: Thaw Medium 2 (BPS Cat. #60184)
- LIGHT, His-Tag (Human) (BPS Cat. #71266)
- LIGHT-CHO Recombinant Cell Line (BPS Cat. # 79262)
- 96-well tissue culture-treated white clear-bottom assay plate
- One-Step luciferase assay system (BPS bioscience # 60690) or other luciferase reagents for measuring firefly luciferase activity
- Luminometer

Assay Protocol

1. Harvest HVEM/NFκB Reporter_Jurkat Recombinant Cell Line (effector cells) from culture in growth medium and seed cells at a density of 35,000 cells per well into white clear-bottom 96-well microplate in 90 µl of assay medium.
2. Dilute the ligand (LIGHT or LIGHT/CHO cells) and agonist/antagonist (e.g. anti-LIGHT or anto-HVEM Ab) in assay medium. Add 10 µl of diluted ligand and antibody to the wells. Add 10 µl of assay medium to control wells. Add 100 µl of assay medium to cell-free control wells (for determining background luminescence). Set up each treatment in at least triplicate.
3. Incubate the plate at 37°C in a CO_2 incubator for 6 hours.
4. Perform luciferase assay using the ONE-Step luciferase assay system: Add 100 µl of One-Step Luciferase reagent per well and rock at room temperature for ~30 minutes. Measure luminescence using a luminometer. *If using luciferase reagents from other vendors, follow the manufacturer's assay protocol.*
5. Data Analysis: Obtain the background-subtracted luminescence by subtracting the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression = background-subtracted luminescence of stimulated well / average background-subtracted luminescence of unstimulated control wells.

Figure 2. HVEM/NFκB-Luc Reporter activities stimulated by different ligands
The results are shown as fold induction of NF-κB luciferase reporter expression.

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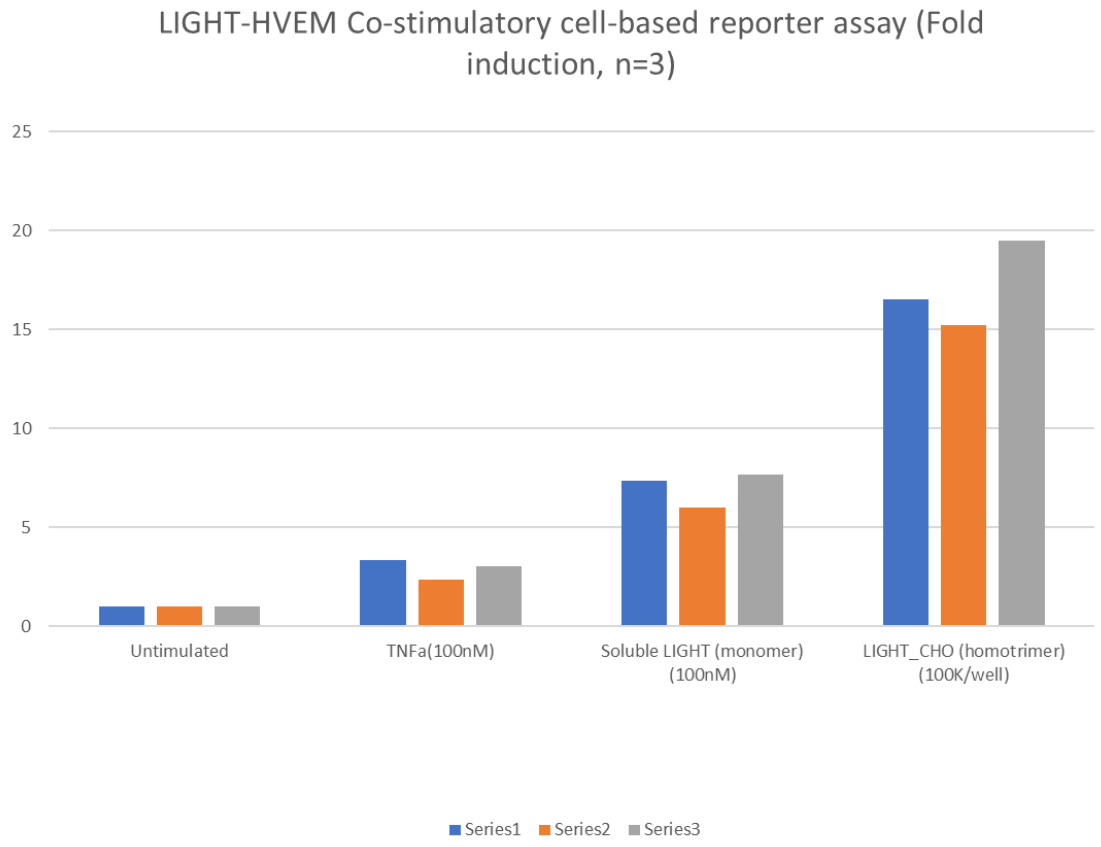

Stimulation by	Fold induction
TNFa	5.3136
sLIGHT	7.6818
LIGH_CHO	19.456

Figure 3. Dose Response of HVEM/NFκB-Luc Reporter activities stimulated by different soluble LIGHT and LIGHT expresses on CHO cell surface

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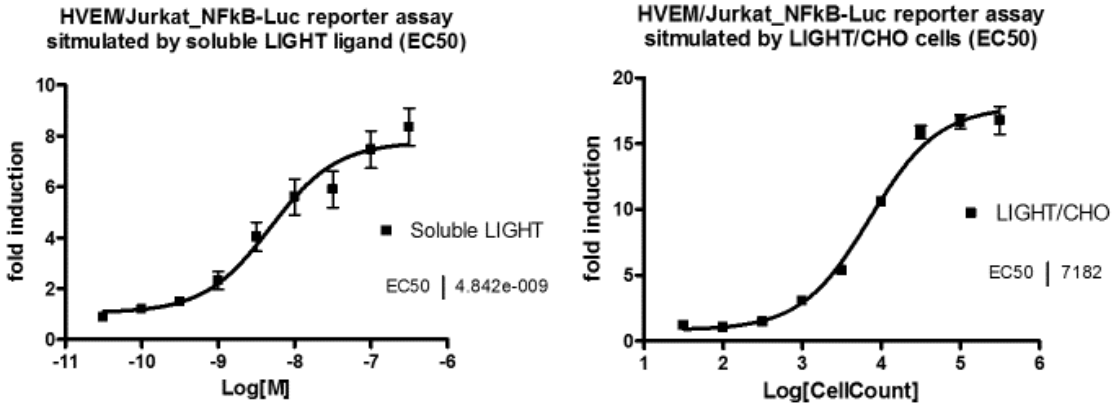
The results are shown as fold induction of NF-κB luciferase reporter expression.

LIGHT-HVEM reporter assay dose response curves



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Related Products

	Cat. #	Size
ONE-Step™ Luciferase Assay System	60690-1	10 ml

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ONE-Step™ Luciferase Assay System	60690-2	100 ml
LIGHT, His-Tag (Human)	71266	100 µg
BTLA:HVEM[Biotinylated] Inhibitor Screening Assay Kit	72008	96 rxns
Thaw Medium 2	60184	100 ml
LIGHT-CHO Recombinant Cell Line	79262	2 vials