Description

LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line is a Jurkat cell line designed for monitoring HVEM (Herpes virus entry mediator) related NF-κB (nuclear factor κB) signal transduction pathways. It contains a firefly luciferase reporter driven by four copies of the NF-κB response element and expresses human HVEM. After activation by the HVEM ligand LIGHT, endogenous NF-κB transcription factors bind to the DNA response elements, inducing transcription of the luciferase reporter.

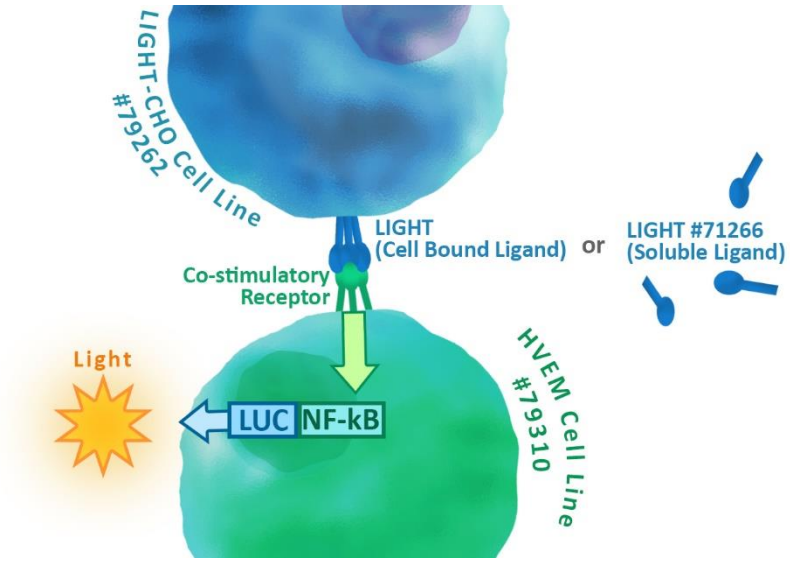

Figure 1: Mechanism of action of LIGHT-Responsive HVEM/NF-κB Jurkat Cell Line in response to LIGHT.
LIGHT, either as a soluble ligand or when present on the cell surface of a cell, binds to HVEM, and NF-κB transcription factors is activated, inducing transcription of the luciferase reporter.

Background

HVEM (Herpes virus entry mediator), also known as CD270 or TNFRSF14, is a human cell surface receptor of the TNF-receptor superfamily that can act as both a co-stimulatory and a co-inhibitory receptor of T cells. Binding of HVEM to one of its ligands, LIGHT (CD258, TNFSF14) or LTα (lymphotoxin-α), causes a co-stimulatory signal which can activate lymphoid cells. Alternately, interaction with BTLA (CD272) or CD160 causes a co-inhibitory signal which negatively regulates T-cell immune responses. HVEM has also been shown to interact with the adaptor proteins TRAF2 and TRAF5 and is critical to herpes simplex virus (HSV) cellular entry. LIGHT, either in the soluble form or as oligomers expressed on the cell surface, can activate NF-κB signaling pathway through binding to HVEM. The LIGHT/HVEM axis are co-stimulatory immune checkpoint molecules extensively studied in cancer immunotherapy.

Application(s)

- Screen for agonists or antagonists of LIGHT-HVEM signaling in a physiologically relevant cellular model.
- Characterize T cell-mediated immune responses of HVEM and its interactions with LIGHT.
- Screen co-stimulatory immune checkpoint molecules for cancer immunotherapy.

Materials Provided

Components	Format
2 vials of frozen cells	Each vial contains >1 x 10^6 cells in 1 ml of Cell Freezing Medium (BPS Bioscience, #79796)

BPS Bioscience
● bpsbioscience.com ● 858-202-1401 ● support@bpsbioscience.com

Host Cell
Jurkat cells (clone E6-1), Human T lymphoblast, suspension.

Mycoplasma Testing
The cell line has been screened to confirm the absence of Mycoplasma species.

Materials Required but Not Supplied

These materials are not supplied with this cell line but are necessary for cell culture and cellular assays. BPS Bioscience reagents systems are validated and optimized for use with this cell line and are highly recommended for best results. Media components are provided in the Media Formulations section.

Materials Required for Cell Culture

Name	Ordering Information
Thaw Medium 2	BPS Bioscience #60184
Growth Medium 2A	BPS Bioscience #60190

Materials Required for Cellular Assay

Name	Ordering Information
Assay Medium: Thaw Medium 2	BPS Bioscience #60184
LIGHT, His-Tag (Human) Recombinant	BPS Bioscience #71266
LIGHT-CHO Recombinant Cell Line	BPS Bioscience #79262
IKK-16 dihydrochloride: inhibitor of NF-κB activation	Sigma #SML1138
NF-κB Luciferase Reporter Jurkat Cell Line	BPS Bioscience #60651
96-well tissue culture-treated white clear-bottom assay plate	Corning #3610
ONE-Step™ luciferase assay system	BPS Bioscience #60690
Luminometer	

Storage Conditions

Cells will arrive upon dry ice and should immediately be thawed or stored in liquid nitrogen upon receipt. Do not use a -80°C freezer for long term storage. Contact technical support at support@bpsbioscience.com if the cells are not frozen in dry ice upon arrival.

Media Formulations
For best results, the use of validated and optimized media from BPS Bioscience is *highly recommended*. To formulate a comparable but not BPS validated media, formulation components can be found below.

Note: Thaw Media does *not* contain selective antibiotics. However, Growth Media *does* contain selective antibiotics, which are used to maintain selective pressure on the cell population expressing the gene of interest. Cells should be grown at 37°C with 5% CO_2. BPS Bioscience's cell lines are stable for at least 10 passages when grown under proper conditions.

Media Required for Cell Culture

Thaw Medium (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Growth Medium 2A (BPS Bioscience #60190):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin plus 1 mg/ml of Geneticin, and 200 µg/ml of Hygromycin B.

Media Required for Functional Cellular Assay

Thaw Medium (BPS Bioscience #60184):
RPMI 1640 medium supplemented with 10% FBS, 1% Penicillin/Streptomycin.

Cell Culture Protocol

Note: Jurkat cells are derived from human material and thus the use of adequate safety precautions is recommended.

Cell Thawing

1. Swirl the vial of frozen cells for approximately 60 seconds in a 37°C water bath. As soon as the cells are thawed (it may be slightly faster or slower than 60 seconds), quickly transfer the entire contents of the vial to a tube containing 10 ml of pre-warmed Thaw Medium 2**.**

   Note: Leaving the cells in the water bath at 37°C for too long will result in rapid loss of viability.

2. Immediately spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cells in 5 ml of pre-warmed Thaw Medium 2**.**

3. Transfer the resuspended cells to a T25 flask and incubate at 37°C in a 5% CO_2 incubator.

4. After 24 hours of culture, check for cell viability. For a T25 flask, add 3-4 ml of Thaw Medium 2 and continue growing in a 5% CO_2 incubator at 37°C until the cells are ready to passage.

5. Cells should be passaged before they reach a density of 2 x 10^6 cells/ml. At first passage and subsequent passages, use Growth Medium 2A.

Cell Passage

Dilute the cell suspension into new culture vessels before they reach a density of 2 x 10^6 cells/ml, but no less than 1 x 10^6 cells/ml in Growth Medium 2A**.** The sub-cultivation ratio recommended is 1:10 to 1:20 twice a week.

Cell Freezing

1. Spin down the cells at 300 *x g* for 5 minutes, remove the medium and resuspend the cell pellet in 4°C Cell Freezing Medium (BPS Bioscience #79796) at a density of ~4 x 10^6 cells/ml.

2. Dispense 1 ml of cell suspension into each cryogenic vial. Place the vials in an insulated container for slow cooling and store at -80°C overnight.

3. Transfer the vials to liquid nitrogen the next day for long term storage.

It is recommended to expand the cells and freeze down at least 10 vials of cells at an early passage for future use.

Validation Data

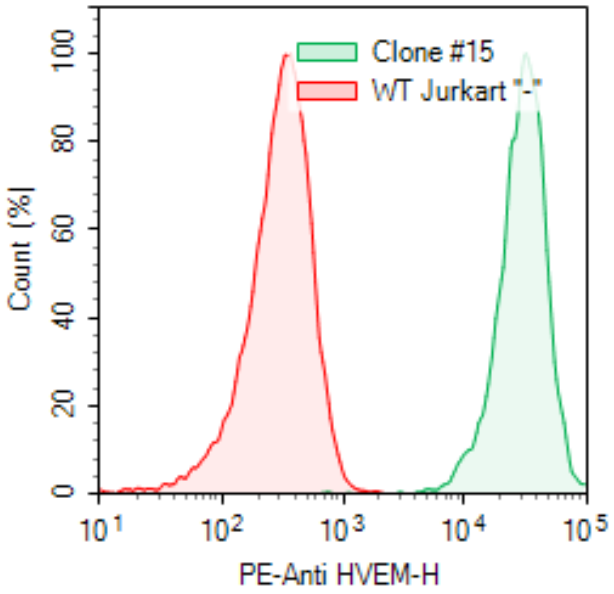

Figure 2: HVEM expression in LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line analyzed by flow cytometry.
LIGHT-Responsive HVEM/NF-κB Reporter Jurkat cells (green) were stained with PE anti-human CD270 (HVEM, TR2) Antibody (BioLegend #318806). Parental Jurkat cells (red) were used as negative control. The y axis represents the % of cells. The x axis indicates PE intensity.

Functional Validation

- The following assay was designed for a 96-well format. To perform the assay in different tissue culture formats, the cell number and reagent volume should be scaled appropriately.
- All conditions should be performed in triplicate.
- Assay A should include "Stimulated", "Background Control" and "Unstimulated Control" conditions.
- Assay B should include "Background Control", "No Inhibitor Control", "No Inhibitor, No Agonist Control" and "Test Inhibitor" conditions.
- We recommend including the NF-κB Luciferase Reporter Jurkat Cell Line as a control.

Note: Use Thaw Medium 2 (BPS Bioscience #60184) as Assay Medium.

A. Response of LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line to agonists

1. Seed LIGHT-Responsive HVEM/NF-κB Reporter Jurkat cells at a density of 35,000 cells per well into clear-bottom white 96-well plate in 50 µl of Assay Medium. Leave empty wells as cell-free control wells ("Background Control").

2. Prepare serial dilutions of agonist (for example, soluble LIGHT, or membrane bound as in the LIGHT-CHO Recombinant Cell Line) at a concentration 2-fold higher than the desired final concentration in Assay Medium (50 µl/well).

3. Add 50 µl of diluted agonist to the "Stimulated" wells.

4. Add 50 µl of Assay Medium to the "Unstimulated Control" wells (for measuring uninduced level of NF-κB reporter activity).

5. Add 100 µl of Assay Medium to "Background Control".

6. Incubate at 37°C with 5% CO_2 for 5-6 hours.

7. Add 100 µl of ONE-Step™ Luciferase reagent per well.

8. Rock gently at room temperature for ~15-20 minutes.

9. Measure luminescence using a luminometer.

10. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The fold induction of NF-κB luciferase reporter expression is the background-subtracted luminescence of treated cells divided by the average background-subtracted luminescence of untreated control cells.

$$Fold\ induction = \frac{average\ Lum\ of\ treated\ cells - average\ background}{average\ Lum\ of\ control\ cells - average\ background}$$

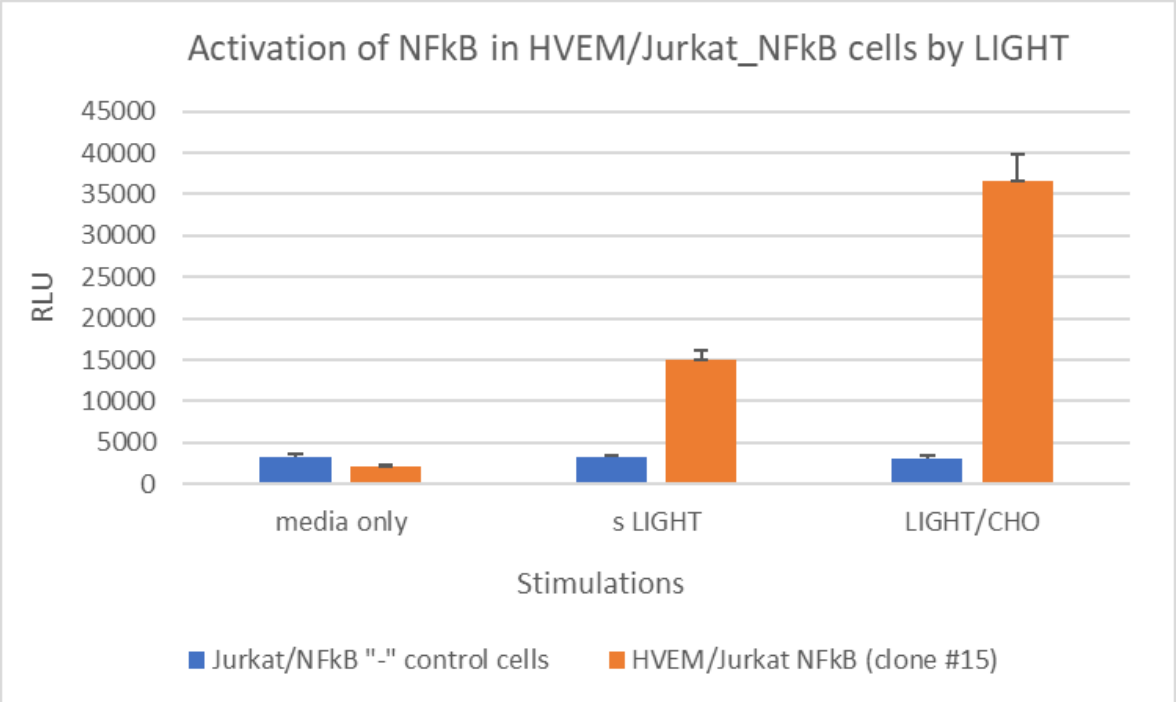

Figure 3. LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line activation by soluble and membrane bound LIGHT.
LIGHT-Responsive HVEM/NF-κB Reporter Jurkat cells and NF-κB Reporter Jurkat cells were incubated with soluble LIGHT and membrane bound LIGHT (LIGHT-CHO Recombinant Cell Line) for 6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System.

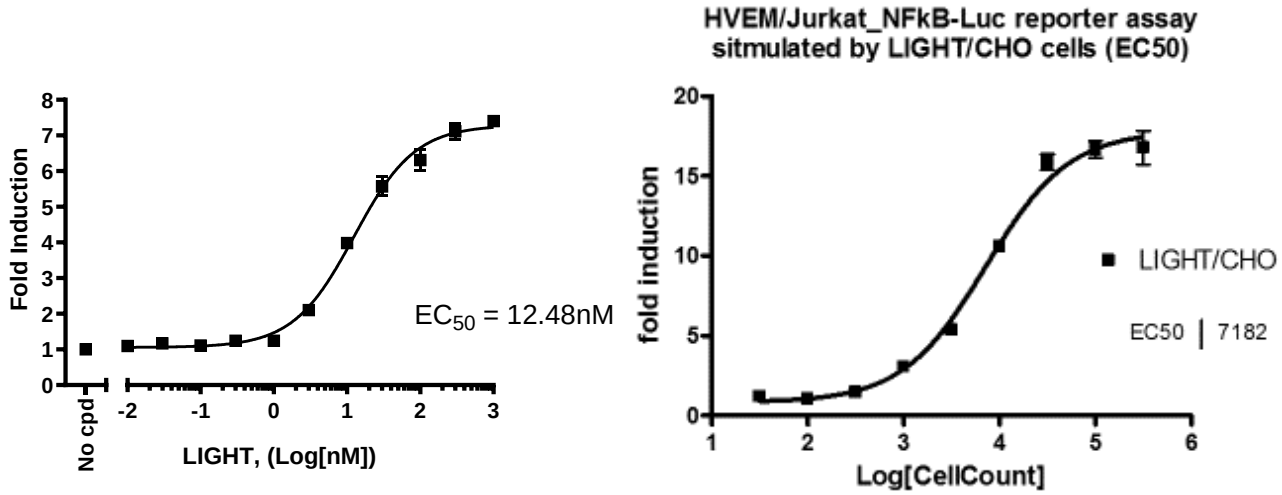

Figure 4. Dose response curves of LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line to soluble and membrane bound LIGHT.
LIGHT-Responsive HVEM/NF-κB Reporter Jurkat cells were incubated with increasing concentrations of soluble LIGHT ligand (top panel) and membrane bound LIGHT (LIGHT-CHO Recombinant Cell Line, bottom panel) 6 hours before measuring luciferase activity with ONE-Step™ Luciferase Assay System.

B. Inhibition of LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line

1. Seed LIGHT-Responsive HVEM/NF-κB Reporter Jurkat cells at a density of 35,000 cells per well into clear-bottom white 96-well plate in 50 µl of Assay Medium. Leave empty wells as cell-free control wells ("Background Control").

2. Prepare a serial dilution of inhibitor at concentrations 4-fold higher than the desired final concentration in Assay Medium (25 µl/well).

3. Add 25 ml of inhibitor serial dilution to the "Test Inhibitor" wells.

4. Add 25 ml of Assay Medium to the "No Inhibitor" and "No Inhibitor, No Agonist" wells.

5. Incubate the plate at 37°C with 5% CO_2 for 30 minutes.

6. Prepare a solution of agonist 4-fold higher than the desired final concentration in Assay Medium (25 µl/well).

7. Add 25 ml of agonist to the "Test Inhibitor" and "No Inhibitor" wells.

8. Add 25 ml of Assay Medium to the "No Inhibitor, No Agonist" wells.

9. Add 100 ml Assay Medium to the "Background Control" wells (for determining background luminescence).

10. Incubate the plate at 37°C with 5% CO_2 for 5-6 hours.

11. Add 100 µl of One-Step™ Luciferase reagent per well.

12. Rock at RT for ~15-20 minutes.

13. Measure luminescence using a luminometer.

14. Data Analysis: Subtract the average background luminescence (cell-free control wells) from the luminescence reading of all wells. The percent luminescence of luciferase reporter expression is the background-subtracted luminescence of treated wells divided by the average background-subtracted luminescence of the untreated control wells x 100%.

$$Percent\ Luminescence = \left(\frac{Luminescence\ of\ \ Test\ Inhibitor\ Wells - avg.background}{Avg.Luminescence\ of\ No\ Inhibitor\ Wells - avg.background}\right) x100$$

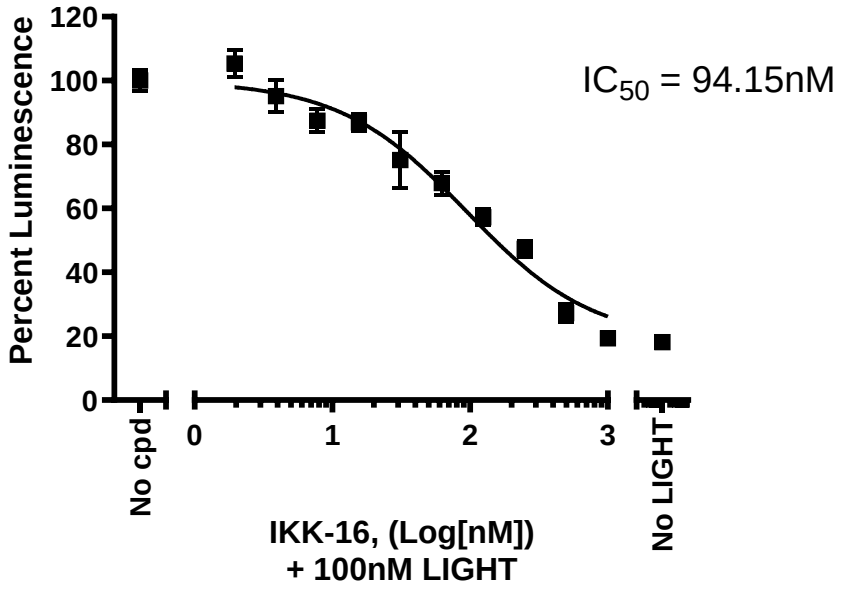

Figure 5: Inhibition of LIGHT-induced HVEM/NF-κB activity by the NF-κB inhibitor IKK-16 dihydrochloride, in LIGHT-Responsive HVEM/NF-κB Reporter Jurkat Cell Line.
LIGHT-Responsive HVEM/NF-κB Reporter Jurkat cells were pre-incubated with increasing doses of IKK-16 prior to stimulation with 100 nM LIGHT, as described in the protocol above. The results are shown as percent luminescence compared to wells without IKK-16 (set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Sequence
Human HVEM (Accession Number NM_003820.2)
MEPPGDWGPPPWRSTPKTDVLRLVLYLTFLGAPCYAPALPSCKEDEYPVGSECCPKCSPGYRVKEACGELTGTVCEPCPPGTYIA
HLNGLSKCLQCQMCDPAMGLRASRNCSRTENAVCGCSPGHFCIVQDGDHCAACRAYATSSPGQRVQKGGTESQDTLCQNCPP
GTFSPNGTLEECQHQTKCSWLVTKAGAGTSSSHWVWWFLSGSLVIVIVCSTVGLIICVKRRKPRGDVVKVIVSVQRKRQEAEGEA
TVIEALQAPPDVTTVAVEETIPSFTGRSPNH

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Troubleshooting Guide

Visit bpsbioscience.com/cell-line-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

References

Chen L. and Flies D., 2013 *Nat Rev Immunol.* 13(4): 227–242.
Steinberg M., *et.al.* 2014 *Immunol Rev.* 244(1): 169–187.
Rio M., *et.al.* 2014 *Transplantation.* 15; 98(11): 1165–1174.
Shui W., *et.al.* 2014 *Immune Netw.* 14(2): 67–72.
Steinberg M., *et.al.* 2009 *Semin. Immunopathol.* 31: 207-221.
Ware C., 2009 *Adv.Exp.Med.Biol.* 647:146.

Related Products

Products	*Catalog #*	*Size*
LIGHT-CHO Recombinant Cell Line	79262	2 vials
LIGHT, His-Tag (Human) Recombinant	71266	100 µg
HVEM, Fc fusion, Biotin-labeled (Human) Recombinant	71143	25 µg/50 µg
HVEM- CHO Recombinant Cell Line	79297	2 vials
HVEM-HEK293 Recombinant Cell Line	79313	2 vials
NF-κB Reporter (Luc) - Jurkat Cell Line	60651	2 vials
NF-κB Reporter Kit (NF-κB Signaling Pathway)	60614	500 reactions

Version 041024