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Data sheet

LIGHT:HVEM[Biotinylated] Inhibitor Screening Assay Kit

Catalog #79684
Size: 96 reactions

BACKGROUND: Herpesvirus entry mediator (HVEM, also known as tumor necrosis factor receptor superfamily member 14 (TNFRSF14) or CD270) and LIGHT (tumor necrosis factor superfamily member 14 (TNFSF14) or CD258) mediate costimulatory responses in T cell activation and proliferation during the immune response. The HVEM/LIGHT co-signaling pathway plays important roles in several biological processes, including autoimmunity, inflammation, bacterial and virus infection, transplant rejection and cancer.

DESCRIPTION: The *LIGHT:HVEM[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of LIGHT:HVEM signaling. This kit comes in a convenient 96-well format, with biotin-labeled HVEM, purified LIGHT, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled HVEM by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, LIGHT is coated on a 96-well plate. Next, HVEM is incubated with LIGHT on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71266	LIGHT, His-Tag (Human)	10 µg	-80 °C	Avoid multiple freeze/thaw cycles!
71143	HVEM, Fc fusion, Biotin-labeled (Human)	5 µg	-80 °C	
79311	3x Immuno Buffer 1	50 ml	-20 °C	
79728	Blocking Buffer 2	50 ml	+4 °C	
79742	Streptavidin-HRP	15 µl	+4 °C	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room Temp	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp	
79699	96-well white microplate	1	+4 °C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Adjustable micropipettor and sterile tips

APPLICATIONS: This kit is useful for screening for inhibitors of HVEM binding to LIGHT.

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STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCES:
Granger, S.W., *et al. Cytokine Growth Factor Rev.* 2003, **14(3-4)**: 289-296
Del Rio, M.L., *et al. J. Leukoc. Biol. 2010,* **87(2)**: 223-235

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with LIGHT:

1) Thaw **LIGHT** on ice. Upon first thaw, briefly spin tube containing **LIGHT** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **LIGHT** in aliquots at -80 °C. Note: **LIGHT** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **LIGHT** to 2 µg/ml in PBS.
3) Add 50 µl of diluted **LIGHT** solution to each well and incubate overnight at 4 °C. Leave a couple of wells empty (uncoated) for use with the "Ligand Control" (see below).
4) Dilute 10 ml **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with 20 ml distilled water. Prepare only enough **1x Immuno Buffer 1** as is required for the assay.
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
6) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature. Remove supernatant as described in step 5.

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1** + 15 µl distilled water)
2) Add 25 µl of master mixture to each well. Use uncoated wells for the "Ligand Control".

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer	10 µl	10 µl	10 µl	10 µl
Distilled water	15 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
HVEM-biotin (1 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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3) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Ligand Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer). Incubate at room temperature for one hour.

4) Thaw **HVEM-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **HVEM-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80 °C. Note: ***HVEM-biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute **HVEM-biotin** to 1 ng/µl (approximately 25 nM) in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 20 µl of **1x Immuno Buffer 1** to the well designated "Blank".

7) Initiate reaction by adding 20 µl of diluted **HVEM-biotin** (see Step 1-5) to wells labeled "Positive Control", "Ligand Control" and "Test Inhibitor". Incubate at room temperature for two hours.

8) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-8.

Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.

2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towel to remove liquid.

4) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

5) Just before use, mix on ice 50 µl **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.

6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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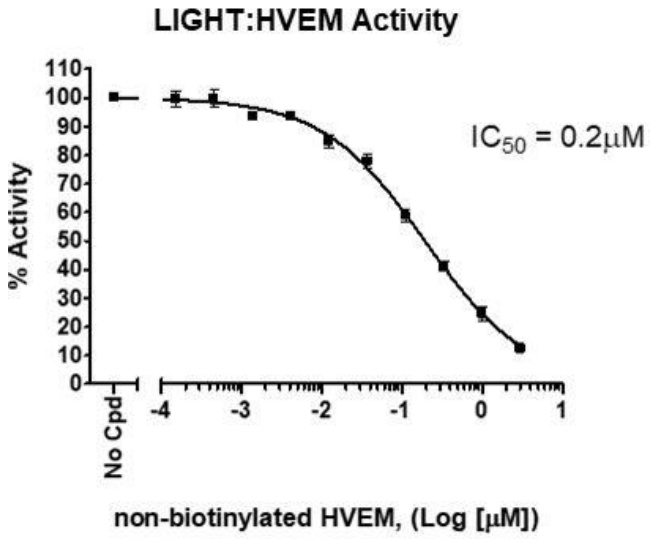
Reading Chemiluminescence:

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second; delay after plate movement is 100 mseconds. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 1000).

Example of assay results:



LIGHT:HVEM binding activity, measured using the using the *LIGHT:HVEM[Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience #79684. Inhibition of LIGHT:HVEM binding using non-biotinylated HVEM, BPS Bioscience #71143 and the *LIGHT:HVEM[Biotinylated] Inhibitor Screening Assay Kit* (right). Luminescence was measured using a Bio-Tek fluorescent microplate reader. Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.

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RELATED PRODUCTS:

Product Name	Catalog#	Size
LIGHT, His-Tag (Human)	71266	100 µg
LIGHT, His-Tag (Mouse)	79068	100 µg
BTLA(CD272), Fc fusion (Human)	71141	100 µg
HVEM, Fc fusion (Human)	71142	100 µg
HVEM, Fc fusion, Biotin-labeled (Human)	71143	50 µg
BTLA, Fc Fusion, Avi-Tag HiP™	100033	100 µg
BTLA, Fc Fusion, Avi-Tag, Biotin-Labeled HiP™	100047	50 µg
CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72007	96 reactions
BTLA:HVEM[Biotinylated] Inhibitor Screening Assay Kit	72008	96 reactions
CTLA4:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72009	96 reactions
LIGHT-CHO Recombinant Cell Line	79262	2 vials
HVEM/NF-κB Reporter Jurkat Recombinant Cell Line	79310	2 vials
HVEM - CHO Recombinant Cell Line	79297	2 vials
HVEM/TCR Activator - CHO Recombinant Cell Line	79551	2 vials
HVEM - HEK293 Recombinant Cell Line	79313	2 vials
BTLA / NFAT - Luciferase Reporter - Jurkat Cell Line	79476	2 vials

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	LIGHT or HVEM has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh HVEM-biotin, (BPS Bioscience #71143) and fresh LIGHT (BPS Bioscience #71266). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of HVEM-Biotin (BPS Bioscience #71143) to create a standard curve