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Data Sheet

BTLA:HVEM[Biotinylated] Inhibitor Screening Assay Kit

Catalog # 72008
Size: 96 reactions

DESCRIPTION: The binding of BTLA to the HVEM receptor (herpesvirus entry mediator, also known as TNFRSF14) triggers cell signaling pathways involved in the negative regulation of T cells. BTLA:HVEM interaction plays a key role in the regulation of inflammatory, autoimmune, and antitumor responses, and is an important target for drug discovery. The *BTLA:HVEM[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of BTLA:HVEM signaling. This kit comes in a convenient 96-well format, with biotin-labeled HVEM, purified BTLA, 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, BTLA is coated on a 96-well plate. Next, HVEM is incubated with BTLA 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	
71141	BTLA*	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71143	HVEM, Biotin-labeled*	5 µg	-80°C	
79742	Streptavidin-HRP	10 µl	+4°C	
79311	3x Immuno Buffer	50 ml	-20°C	
79728	Blocking buffer 2	50 ml	+4°C	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room Temp	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp	
79699	White 96-well microplate	1	+4°C	

**The concentrations of BTLA and HVEM are lot-specific and will be indicated on the tubes containing the protein.*

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

PBS (Phosphate buffered saline)
Luminometer or microplate reader capable of reading chemiluminescence
Rotating or rocker platform

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

STABILITY: One year from date of receipt when stored as directed.

REFERENCES:

1. Derre L., *et al*. *J. Clin. Invest.* 2010, **120(1):** 157-167.
2. Steinberg, M.W., *et al. J. Exp.Med.* 2008, **205(6):** 1463-1476.

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

Coating the plate with BTLA:

1) Thaw **BTLA** on ice. Upon first thaw, briefly spin tube containing **BTLA** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **BTLA** in aliquots at -80ºC. *Note: **BTLA** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **BTLA** to 2 ng/µl in PBS.

3) Add 50 µl of diluted **BTLA** solution to each well and incubate overnight at 4ºC. Leave a couple of wells empty (uncoated), for use with the "Ligand Control" (see below).

4) Dilute **3x Immuno Buffer** to **1x Immuno Buffer** with water.

5) Decant to remove supernatant. Wash the plate 3 times with 100 µl **1x Immuno Buffer**. 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** + 15 µl H_2O).

2) Add 25 µl of master mixture to each well. Use uncoated wells for the "Ligand Control".

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.

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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 protein.*

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer	10 µl	10 µl	10 µl	10 µl
H_2O	15 µl	15 µl	15 µl	15 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x Immuno Buffer	20 µl	–	–	–
HVEM-biotin (1 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

6) Add 20 µl of **1x Immuno Buffer** 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 three times with 100 µl/well **1x Immuno Buffer**. 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**. Tap plate onto clean paper towels to remove liquid.

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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 ECCL 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.

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 msec. 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 100).

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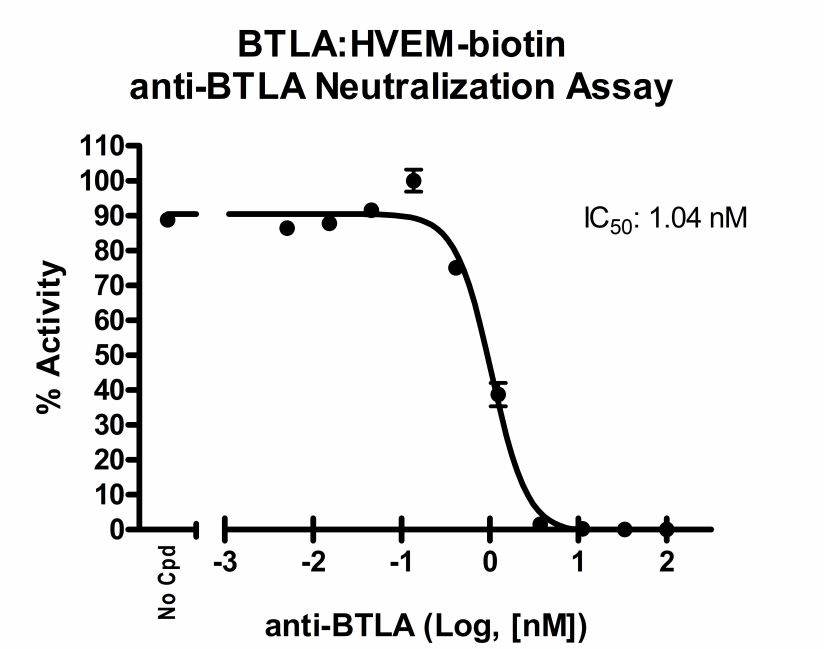
Example of Assay Results:



Inhibition of BTLA:HVEM interaction by BTLA antagonist antibody (BPS Bioscience, #100244). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
HVEM	71142	100 µg
HVEM, Biotin labeled	71143	50 µg
BTLA	71141	100 µg
BTLA Antagonist Antibody	100244	
CD28	71113	200 µg
B7-1	71125	100 µg
B7-1, Biotin labeled	71114	50 µg
CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72007	96 rxns
PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 rxns
PD-L1 Inhibitor Screening Assay Kit	72005	96 rxns
PD-L2 Inhibitor Screening Assay Kit	72006	96 rxns
PD-1	71106	100 µg
PD-1, Biotin labeled	71109	50 µg

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PD-L1	71104	100 µg
PD-L1, Biotin-labeled	71105	50 µg
PD-L2	71107	100 µg
PD-L2, Biotin-labeled	71108	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	HVEM or BTLA has lost activity	Protein loses binding activity upon repeated freeze/thaw cycles. Use fresh HVEM-biotin, (BPS Bioscience #71143) and fresh BTLA (BPS Bioscience #71141). Store proteins in single-use aliquots. Increase time of incubation. Increase protein 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.