6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

CD40:CD40L[Biotinylated] Inhibitor Screening Assay Kit

Catalog # 79257
Size: 96 reactions

DESCRIPTION:

The *CD40:CD40L[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of CD40:CD40L signaling. This kit comes in a convenient 96-well format, with biotin-labeled CD40, purified CD40L, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled CD40 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, CD40L is coated on a 96-well plate. Next, CD40 is incubated with CD40L 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	
79102	CD40, Biotin-labeled	50 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71191	CD40L-His	10 µg	-80°C	
79742	Streptavidin-HRP	15 µl	+4°C	
79311	3x Immuno Buffer 1	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	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

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

APPLICATIONS: This kit is useful for screening for inhibitors of CD40L binding to CD40.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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

REFERENCES:

1. Lutgens, E., *et al., Nat. Med*. 1998; **5:** 1313-1316.
2. Quezada, S.A., *et al., Immunology*. 2003; **22:** 307-328

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

Coating the plate with CD40L:

1) Thaw **CD40L** on ice. Upon first thaw, briefly spin tube containing **CD40L** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining CD40L in aliquots at -80°C. *Note: CD40L is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **CD40L** to 2 µg/ml in PBS.

3) Add 50 µl of diluted **CD40L** 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 **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water. Dilute only enough **3x Immuno Buffer 1** required for the assay and washing the plate; store remainder at -20°C.

5) Decant to remove supernatant. Wash the plate 3 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 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.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

4) Thaw **CD40-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **CD40-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. *Note: CD40-biotin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Blank	Ligand Control	Positive Control	Test Inhibitor
3× Immuno Buffer 1	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	–
1× Immuno Buffer 1	20 µl	–	–	–
CD40-biotin (22.5 µg/ml)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Dilute **CD40-biotin** to 22.5 µg/ml 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 wells designated “Blank”.

7) Initiate reaction by adding 20 µl of diluted **CD40-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 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 towels to remove liquid.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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.

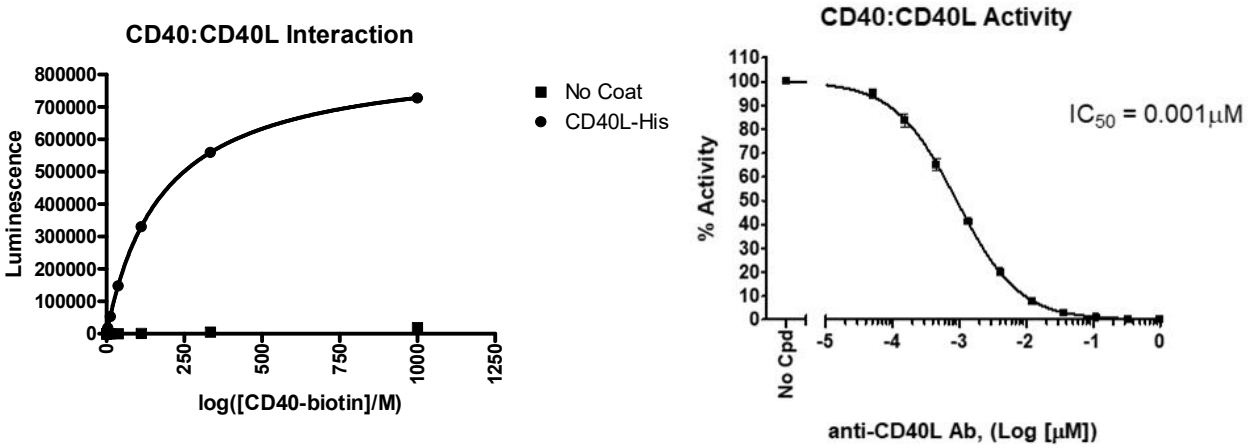
Reading Chemiluminescence:

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).

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Example of Assay Results:



(Left) Interaction of CD40L with CD40-biotin using the *CD40L:CD40[Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience, #79257. (Right) Inhibition of CD40L:CD40 interaction by anti-CD40 antibody (BioLegend, #310827). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
CD40, Fc fusion (Human)	71174	100 µg
CD40, Fc Fusion (IgG1) Avi-Tag, Biotin Labeled	79102	50 µg
CD40L (CD154), His-tag (Human)	71191	100 µg
CD40 - HEK293 Cell Line	71257	2 vials
CD40/NF-κB Reporter (Luc) - HEK293 Cell Line	60626	2 vials
CD40:CD40L TR-FRET Assay	79258	384 rxns.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	CD40 or CD40L has lost activity	Proteins loses functionality upon repeated freeze/thaw cycles. Use fresh CD40-biotin, (BPS Bioscience #79102) and fresh CD40L (BPS Bioscience #71190). Store proteins in single-use aliquots. Increase time of protein 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 protein incubation.
	Results are outside the linear range of the assay	Use different concentrations of CD40-biotin (BPS Bioscience #79102) to create a standard curve.