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Data Sheet

CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit

Catalog # 72007
Size: 96 reactions

DESCRIPTION: The activation of naïve T cells requires two signals, the specific T cell receptor recognition of MHC/Antigen on the surface of the antigen-presenting cell (APC), and the binding of B7-1 (CD80) ligand on the APC with the CD28 receptor on the T cell surface. CD28:B7-1 interaction is an important drug target for the regulation of T cells involved in autoimmunity, inflammation, tumor recognition, and immune tolerance. The *CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of CD28:B7-1 signaling. This kit comes in a convenient 96-well format, with biotin-labeled B7-1, purified CD28, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled B7-1 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, CD28 is coated on a 96-well plate. Next, B7-1 is incubated with CD28 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	
71114	B7−1, Biotin-labeled	2 x 5 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71113	CD28	10 µg	-80°C	
79742	Streptavidin-HRP	10 µl	+4°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4°C	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4°C	
79699	White 96-well microplate	1	+4°C	

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or microplate reader capable of reading chemiluminescence
Rotating or rocker platform

APPLICATIONS: This kit is useful for screening for inhibitors of B7-1 binding to CD28.

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

REFERENCES:

1. Keir, M.E., *et al*. *Immunol. Rev.* 2005, **204:** 128-143.
2. Haas, C., *et al. Int. J. Cancer.* 2006, **1;118:** 658-667.

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

Coating the plate with CD28:

1) Thaw **CD28** on ice. Upon first thaw, briefly spin tube containing **CD28** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining CD28 in aliquots at -80°C. *Note: CD28 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **CD28** to 2 µg/ml in PBS.

3) Add 50 µl of diluted **CD28** 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.

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 4.

Step 1:
1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1**+ 15 µl H_2O).

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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.

4) Thaw **B7-1-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **B7-1-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. *Note: B7-1-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	–	–	–
B7-1-biotin (5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Dilute **B7-1-biotin** to 5 ng/µl 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 **B7-1-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.

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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.

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 **HRP Chemiluminescent Substrate A** and 50 µl **HRP Chemiluminescent 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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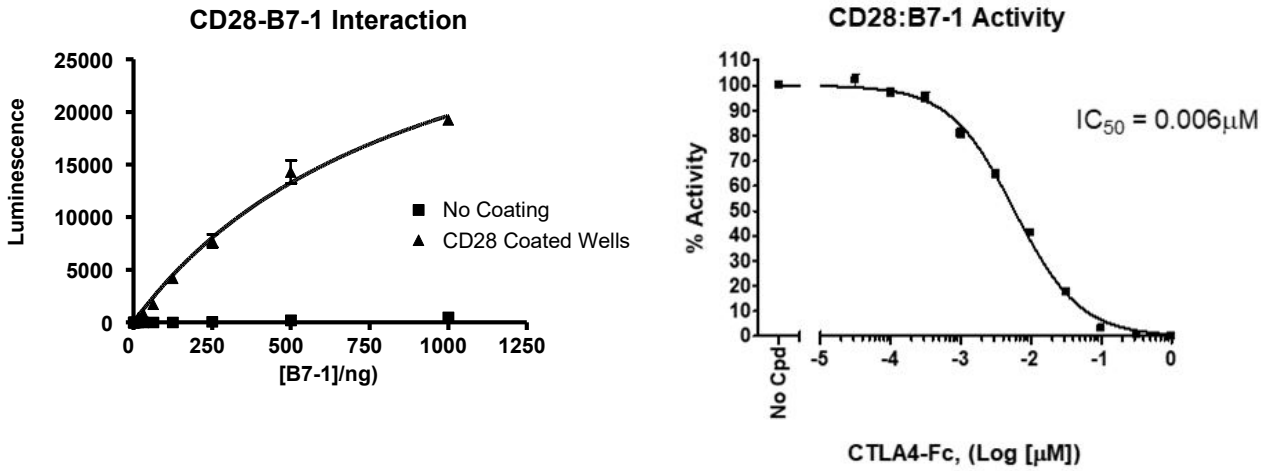
Example of Assay Results:



(Left) CD28:B7-1 binding activity, measured using the using the *CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog #72007. Luminescence was measured using a Bio-Tek fluorescent microplate reader. (Right) Inhibition of CD28:B7-1 interaction by CTLA4-Fc. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
CD28	71113	200 µg
B7-1, Biotin labeled	71114	50 µg
B7-1	71125	100 µg
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
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	CD28 or B7-1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh B7-1-biotin, (BPS Bioscience #71114) and fresh CD28 (BPS Bioscience #71113). 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 B7-1-biotin (BPS Bioscience #71114) to create a standard curve.