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Data Sheet

CTLA4 [Biotinylated]:B7-1 Inhibitor Screening Assay Kit

Catalog #79973
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. Conversely, binding of CTLA4 to B7-1 on the T-cell surface results in an inhibitory signal and prevents T-cell activation. CTLA4B7-1 interaction is an important drug target for the regulation of the host's response to cancer. The *CTLA4 [Biotinylated]:B7-1 Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of CTLA4:B7-1 signaling. This kit comes in a convenient 96-well format, with biotin-labeled CTLA4 (CTLA4[B]), purified B7-1, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled CTLA4 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, B7-1 is coated on a 96-well plate. Next, CTLA4[B] is incubated with B7-1 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	
71125	B7−1	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71152	CTLA4, Biotin-labeled	>1 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	
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	

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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 B7-1 binding to CTLA4.

STABILITY: 6 months from date of receipt when stored as directed.

REFERENCES:
1. Linsley, P.S., *et al. J. Exp. Med.* 1991, **174(3):** 561-569.
2. Hurwitz, A.A., *et al. Canc. Res.* 2000, **60:** 2444-2448.

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

Coating the plate with B7-1:

1) Thaw **B7-1** on ice. Upon first thaw, briefly spin tube containing **B7-1** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **B7-1** in aliquots at -80°C. *Note: **B7-1** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **B7-1** to 2 µg/ml in PBS.

3) Add 50 µl of diluted **B7-1** 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. Reserve some undiluted **3x Immuno Buffer 1** for use in later steps of the assay (below).

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.

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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) Prepare the test inhibitor solution.

   The final concentration of DMSO in the assay should not exceed 1%. If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in water (at this step the compound concentration is 10-fold higher than the final concentration).

   If the inhibitor compound is soluble in water, make an aqueous solution of the compound 10-fold higher than the final concentration.

4) Add 5 µl of test inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control" and "Blank," add 5 µl of inhibitor buffer (water or 10% DMSO in water, depending which inhibitor solution is used). Incubate at room temperature for one hour.

5) Thaw **CTLA4-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **CTLA4-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. *Note: **CTLA4-biotin** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Blank	Positive Control	Test Inhibitor
3x Immuno Buffer 1	10 µl	10 µl	10 µl
H_2O	15 µl	15 µl	15 µl
Test Inhibitor	–	–	5 µl
Inhibitor buffer	5 µl	5 µl	–
1x Immuno Buffer 1	20 µl	–	–
CTLA4-biotin (0.125 ng/µl)	–	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

6) Dilute **CTLA4-biotin** to 0.125 ng/µl in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

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

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8) Initiate reaction by adding 20 µl of diluted **CTLA4-biotin** (see Step 1-5) to wells labeled "Positive Control" and "Test Inhibitor." Incubate at room temperature for two hours.

9) 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.

10) 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 1-9.

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.

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.

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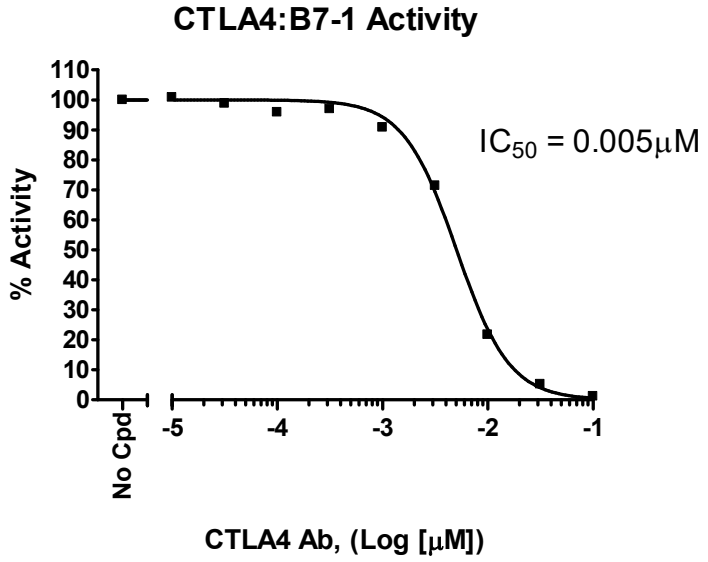
To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second; delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the "hole" position 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).

Example of Assay Results:



Inhibition of CTLA4 [B]:B7-1 binding using the *CTLA4 Neutralizing Antibody,* BPS Bioscience #71212 and the *CTLA4 [Biotinylated]:B7-1 Inhibitor Screening Assay Kit*. 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 support@bpsbioscience.com.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
CTLA4 (CD152), Fc fusion	71149	100 µg
CTLA4 (CD152), Biotin labeled	71152	50 µg
B7-1	71125	100 µg
B7-1, Biotin labeled	71114	50 µg
B7-2	71150	100 µg
CD28	71113	200 µg
CTLA4 (CD152) Neutralizing Antibody	71212	50 µg
CD28:B7-1[Biotinylated] Inhibitor Screening Assay Kit	72007	96 rxns

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	CTLA4 or B7-1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh B7-1, (BPS Bioscience #71125) and fresh biotin-labeled CTLA4 (BPS Bioscience #71152). 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 CTLA4-biotin (BPS Bioscience #71152) to create a standard curve.