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Data Sheet

CD47:SIRP-α[Biotinylated] Inhibitor Screening Assay Kit

Catalog # 72044
Size: 96 reactions

BACKGROUND: Signal-regulatory protein alpha (SIRP-α), also known as CD172a, is a cell surface protein expressed mainly by myeloid cells such as macrophages. Its receptor, CD47, is ubiquitously expressed on the surface of all cells and has been found to be overexpressed in some cancers. CD47 binding to SIRP-α delivers a "Do Not Eat Me" signal to macrophages. In the context of a tumor, this signaling aids in tumor evasion of the immune system. Inhibitors of this interaction hold great promise for cancer immunotherapy.

DESCRIPTION: The *CD47:SIRP-α[Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of CD47:SIRP-α interaction. This kit comes in a convenient 96-well format, with biotin-labeled SIRP-α, purified CD47, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled SIRP-α by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, CD47 is coated on a 96-well plate. Next, SIRP-α-biotin is incubated with CD47 on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71177	CD47, Fc fusion	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71138	SIRP-α (CD172a), His-tag, Biotin-labeled	60 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 microplate reader capable of reading chemiluminescence
Rotating or rocker platform

APPLICATIONS: This kit is useful for screening for inhibitors of CD47 binding to SIRP-α.

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

REFERENCES: Chao, M.P., *et al., Curr. Opin. Immunol.* 2012; **24(2)**: 225-232.

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

Coating the plate with CD47:

1) Thaw **CD47** on ice. Upon first thaw, briefly spin tube containing **CD47** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **CD47** in aliquots at -80ºC. *Note: **CD47** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **CD47** to 2 ng/µl in PBS.

3) Add 50 µl of diluted **CD47** 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 1** to **1x Immuno Buffer 1** in water. Dilute only enough **3x Immuno Buffer 1** required for washing the plate (below).

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 above.

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".

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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.

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x 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	–
1x Immuno Buffer 1	20 µl	–	–	–
SIRP-α-biotin (30 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

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

5) Dilute **SIRP-α-biotin** in **1x Immuno Buffer 1** to 30 ng/µl. 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 **SIRP-α-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.

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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 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 **ELISA ECL Substrate A** and 50 µl **ELISA ECL Substrate B** per well of the reaction, 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 binding partner (typically we set this value as 100).

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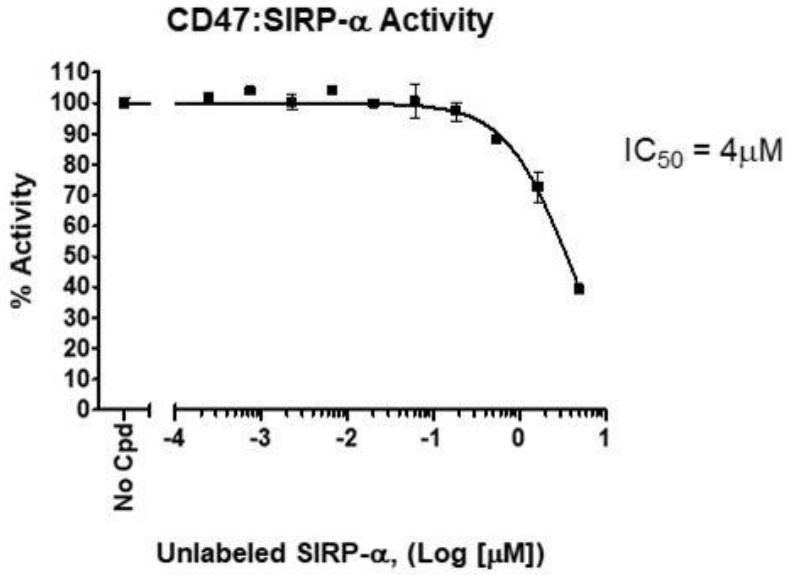
Example of Assay Results:



CD47:SIRP-α-[biotinylated] binding inhibition by unlabeled SIRP-α, measured using the CD47:SIRP-α-[biotinylated] *Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog #72044. 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.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
CD47, Fc fusion	71177	100 µg
CD47, His-tag	71127	100 µg
SIRP-α, His-tag	71145	100 µg
SIRP-α, His-tag, Biotin-labeled	71138	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	CD47 or SIRP-α has lost binding capacity	Protein loses activity upon repeated freeze/thaw cycles. Use fresh SIRP-α-biotin, (BPS Bioscience #71138) and fresh CD47 (BPS Bioscience #71177). Store proteins in single-use aliquots. Increase time of protein incubation. Increase protein concentration.
	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.
	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
Luminescent signal is erratic or varies widely among wells	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Insufficient washes	Increase number of washes. Increase wash volume. Add Tween-20 to 0.1% in washing buffer.
Background (signal to noise ratio) is high	Sample solvent is inhibiting the protein	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 SIRP-α-biotin (BPS Bioscience #71138) to create a standard curve.