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Data Sheet
CD47:SIRP- γ [Biotinylated] Inhibitor Screening Assay Kit
Catalog # 72059
Size: 96 reactions

BACKGROUND: Signal-regulatory protein gamma (SIRP- λ), also known as CD172g, 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- λ has been shown to be important for T cell trans-endothelial migration (TEM).

DESCRIPTION: The *CD47:SIRP- γ [Biotinylated] Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of CD47:SIRP- γ signaling. This kit comes in a convenient 96-well format, with biotin-labeled SIRP- γ (CD172g), 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- γ through 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- γ 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 be measured using a chemiluminescence reader.

COMPONENTS:

| Catalog # | Component | Amount | Storage | |
|-----------|---|------------|---------|---|
| 71236 | SIRP- γ , Biotin-labeled | 5 μ g | -80°C | (Avoid freeze/ thaw cycles!) |
| 71177 | CD47 | 10 μ g | -80°C | |
| 79742 | Streptavidin-HRP | 15 μ l | +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 | RT | |
| | ELISA ECL substrate B (brown bottle) | 6 ml | RT | |
| 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 SIRP- γ binding to CD47.

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

REFERENCES:

1. Stefanidakis, *et al.*, *Blood*. 2008, **112(4)**:1280-1289
2. Sick, E., *et al.* *Br J Pharmacol*. 2012, **167(7)**:1415-1430.

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 -80°C. *Note: CD47 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*
- 2) Dilute **CD47** to 2 $\mu\text{g/ml}$ in PBS.
- 3) Add 50 μl of diluted **CD47** 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 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 4.

Step 1:

- 1) Prepare the master mixture: N wells \times (10 μl **3x Immuno Buffer 1** + 15 μl H₂O).

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- 2) Add 25 μ l of master mixture to each well. Use uncoated wells for the “Ligand Control”.
- 3) Add 5 μ l of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control”, “Ligand Control”, and “Blank”, add 5 μ l of the same solution without inhibitor (inhibitor buffer). Incubate at room temperature for one hour.
- 4) Thaw **SIRP- γ -biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **SIRP- γ -biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C . *Note: SIRP- γ -biotin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

| | Blank | Ligand Control | Positive Control | Test Inhibitor |
|---------------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 3x Immuno Buffer 1 | 10 μ l | 10 μ l | 10 μ l | 10 μ l |
| H ₂ O | 15 μ l | 15 μ l | 15 μ l | 15 μ l |
| Test Inhibitor/Activator | - | - | - | 5 μ l |
| Inhibitor buffer (no inhibitor) | 5 μ l | 5 μ l | 5 μ l | - |
| 1x Immuno Buffer 1 | 20 μ l | - | - | - |
| SIRP- γ -biotin (2 μ g/ml) | - | 20 μ l | 20 μ l | 20 μ l |
| Total | 50 μl | 50 μl | 50 μl | 50 μl |

- 5) Dilute **SIRP- γ -biotin** to 2 μ g/ml in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.
- 6) Add 20 μ l of **1x Immuno Buffer 1** to the well designated “Blank”.
- 7) Initiate reaction by adding 20 μ l of diluted **SIRP- γ -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 μ l/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.
- 9) Block wells by adding 100 μ l 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 μ l 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 μ l of **Blocking Buffer** 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 μ l **ELISA ECL Substrate A** and 50 μ l **ELISA ECL Substrate B**, then add 100 μ l 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 this method uses 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).

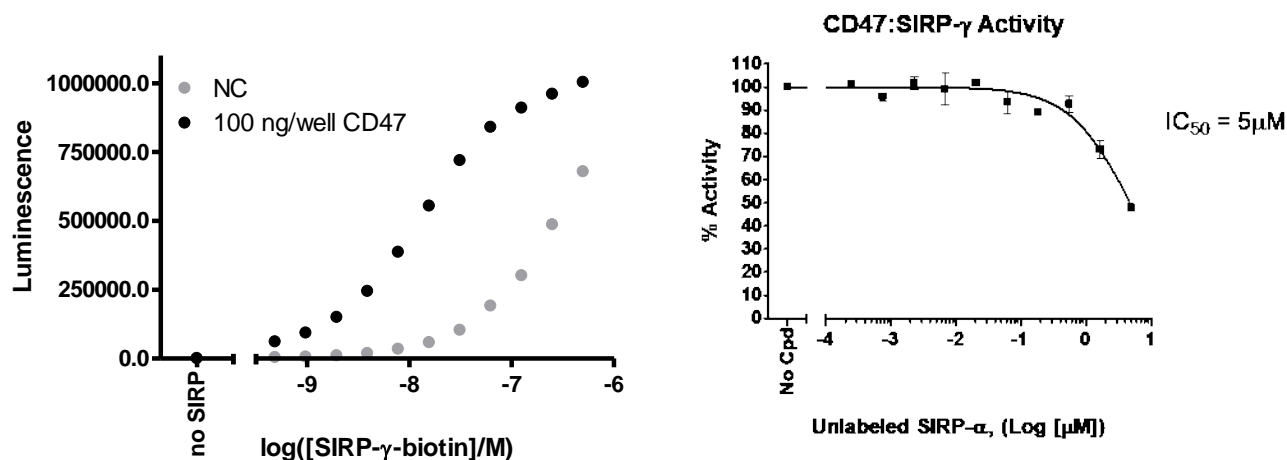
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Example of Assay Results:



(Left) Interaction of CD47 with SIRP- γ -biotin using the *CD47:SIRP- γ [Biotinylated] Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog #72059 (non-coated wells, NC). (Right) Inhibition of CD47:SIRP- γ interaction by SIRP- α . *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

RELATED PRODUCTS:

| <u>Product Name</u> | <u>Catalog #</u> | <u>Size</u> |
|--|------------------|-------------|
| CD47, Fc fusion | 71177 | 100 μ g |
| CD47, Fc fusion, Biotin-labeled | 71169 | 50 μ g |
| CD47, His-tag | 71127 | 100 μ g |
| SIRP- α , His-tag | 71145 | 100 μ g |
| SIRP- α , His-tag, Biotin-labeled | 71138 | 50 μ g |
| SIRP- γ (CD172g), Fc fusion, Biotin-labeled | 71236 | 50 μ g |
| CD47:SIRP- α [Biotinylated] Inhibitor Screening Assay Kit | 72044 | 96 rxns |

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TROUBLESHOOTING GUIDE

| Problem | Possible Cause | Solution |
|--|---|---|
| Luminescence signal of positive control reaction is weak | CD47 or SIRP- γ has lost activity | Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh SIRP- γ -biotin, (BPS Bioscience #71236) and fresh CD47 (BPS Bioscience #71177). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme 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. |
| 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 SIRP- γ -biotin (BPS Bioscience #71236) to create a standard curve. |

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