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Data Sheet

IL-23R:IL-23A[Biotin] Inhibitor Screening Assay Kit

Catalog # 78014
96 Reactions

DESCRIPTION: The *IL-23RA:IL-23A[Biotin] Inhibitor Screening Assay Kit* is designed for screening inhibitors of IL-23A:IL-23RA interaction. The *IL-23RA-IL-23A[Biotin] Binding Assay Kit* comes in a convenient 96-well format, with biotin-labeled IL-23A, purified IL-23 Receptor alpha (IL-23RA), streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled IL-23A by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, IL-23RA is coated on a 96-well plate. Next, IL-23A[Biotin] is incubated with IL-23RA 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.

BACKGROUND: IL-23A is a pro-inflammatory cytokine that plays a key role in inflammation, autoimmunity, and host defense. Due to its role as a mediator of inflammation, the IL-23 pathway has become a popular target for treating diseases that have a strong inflammatory component such as bowel disease and colon cancer.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	IL-23A[Biotin]	2 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	IL-23RA	20 µg	-80°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
79742	Streptavidin-HRP	15 µl	+4°C	
79728	Blocking buffer 2	50 ml	+4°C	
79670	ELISA ECL substrate A (translucent bottle)	6 ml	+4°C	
79670	ELISA ECL substrate B (brown bottle)	6 ml	+4°C	
79699	White 96-well microplate	1	+4°C	

APPLICATIONS: Great for screening small molecules and antibodies that inhibit the binding of IL-17A to IL-17RA.

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS buffer
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Rotating or rocker platform

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

REFERENCES:

1. McKenzie, B.S., *et al*. 2006. "Understanding the IL-23–IL-17 immune pathway." *Trends in Immunology* **27(1):** 17-23.
2. Iwakura, Y., and Harumichi I. 2006. "The IL-23/IL-17 axis in inflammation." *The Journal of clinical investigation* **116(5):** 1218-1222.

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

Coating the plate with IL-23RA:

1) Thaw **IL-23RA** on ice. Upon first thaw, briefly spin tube containing **IL-23RA** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **IL-23RA** in aliquots at -80ºC. *Note: **IL-23RA** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **IL-23RA** to 4 ng/µl in 1x PBS.

3) Add 50 µl of diluted **IL-23RA** 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 10 ml of **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with 20 ml water.

5) Decant to remove supernatant. Wash the plate 3 times using 100 µl **1x Immuno Buffer 1** per well. 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. Decant to remove supernatant.

Step 1:

1) Prepare the master mixture: N wells x (10 µl **3x Immuno Buffer 1** + 15 µl distilled water)

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2) Add 25 µl of master mixture to each well. Use uncoated wells for the "Ligand Control."

	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 (10% DMSO in water)	5 µl	5 µl	5 µl	–
1x Immuno Buffer 1	20 µl	–	–	–
IL-23A[Biotin] (0.5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

3) Prepare the 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 distillled water (at this step the compound concentration is 10-fold higher than the final concentration).

If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration you wish to test.

4) 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, usually 10% DMSO in water). *Note: Preincubation of the inhibitors with the IL-23A-coated plate may be necessary, depending on the mechanism of inhibition*).

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

6) Thaw **IL-23A-biotin** on ice. Upon first thaw, briefly spin tube containing **IL-23A-biotin** protein to recover full contents of the tube. Aliquot **IL-23A-biotin** into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C. *Note: **IL-23A-biotin** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

7) Dilute **IL-23A-biotin** in **1x Immuno Buffer 1** at 0.5 ng/µl (10 ng/20 µl). Keep diluted protein on ice until use. Discard any unused diluted protein after use.

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8) Initiate reaction by adding 20 µl of diluted **IL-23A-biotin** to the wells labeled "Ligand Control," "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 **1x Immuno Buffer 1** per well. 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. Decant to remove supernatant.

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 **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 the protein (typically we set this value as 100).

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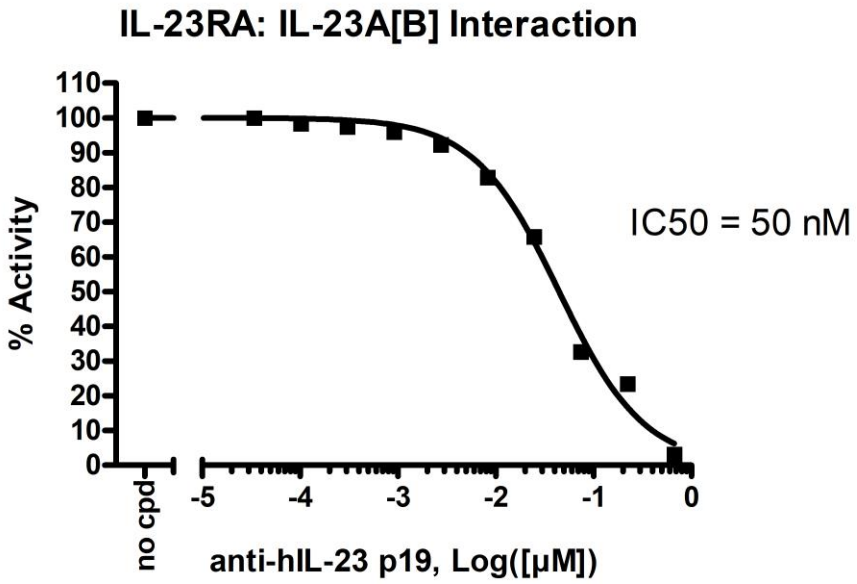
Example of Assay Results:



Inhibition of IL-23RA-IL-23A[B] binding activity by R and D Systems anti-hiL-23 p19 (#AF1716) recognizing the p19 subunit of IL-23, measured using the IL-23RA-IL-23A[Biotin] Binding Assay Kit, BPS Bioscience Catalog #78014 and Anti-hIL-23A Antibody. 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**
ELISA ECL Substrate	79670	200 ml
Streptavidin-HRP	79742	15 µl
Immuno Buffer 1	79311	50 ml
Blocking Buffer 2	79728	50 ml

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	IL-23A or IL-23RA has lost binding activity	Proteins lose binding ability upon repeated freeze/thaw cycles. Use fresh IL-23RA-biotin and fresh IL-23A. Store proteins in single-use aliquots. Increase time of incubation of IL-23A on the plate. Increase IL-23A 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 TBST.
	Sample solvent is inhibiting protein binding	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of protein binding incubation.
	Results are outside the linear range of the assay	Use different concentrations of IL-23RA-biotin to create a standard curve.