Description

The IL-23R:IL-23A[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is designed to measure the binding of IL-23R (interleukin 23 receptor) to IL-23A (IL-23 subunit alpha) for screening and profiling applications. The IL-23R:IL-23A[Biotinylated] Inhibitor Screening Assay Kit comes in a convenient 96-well format, with enough recombinant purified biotinylated IL-23A, IL-23R, blocking and assay buffer and detection reagents for 100 enzyme reactions.

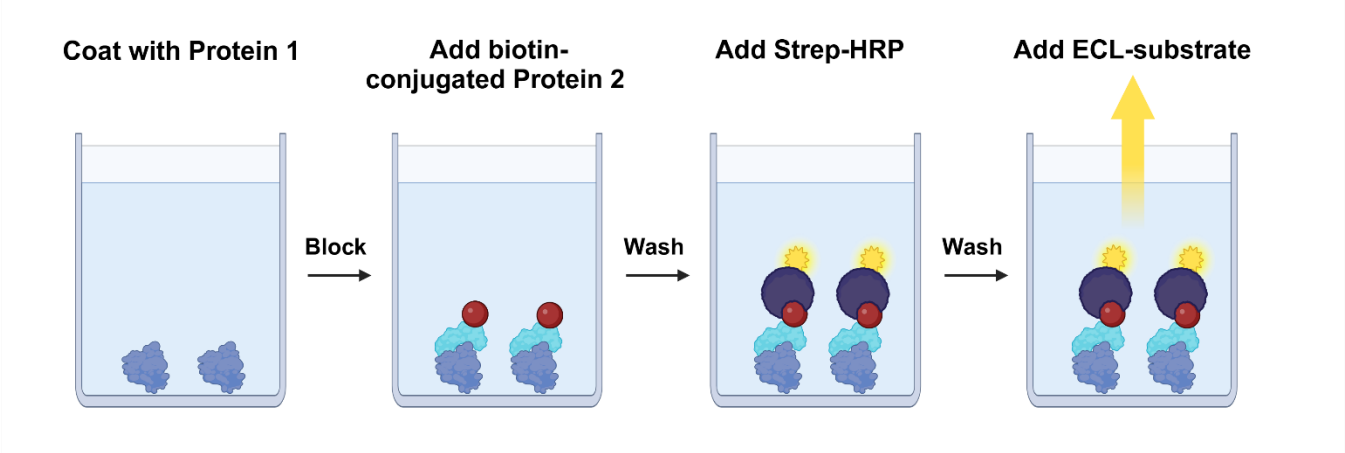

Figure 1. IL-23R:IL-23A[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit schematic.
A 96-well plate is coated with IL-23R protein. After coating and blocking, biotinylated IL-23A added in an optimized assay buffer. Next, unbound biotinylated IL-23A is washed away, and the plate is incubated with streptavidin-HRP. After a final wash, ELISA ECL substrate is added to produce chemiluminescence that can be measured using a chemiluminescence reader. The chemiluminescence signal is proportional to the efficacy of IL-23R binding to IL-23A.

Background

IL-23 (interleukin 23) is a cytokine involved in inflammation. It is composed of two subunits, IL-12B (or IL-12p40) and IL-23A (or IL-23 p19) and binds to the IL-23 receptor. It supports Th17 T cell maintenance, expansion and cytokine release, NK cell IFNγ (interferon γ) secretion and increased ADCC (antibody-dependent cellular cytotoxicity) and $CD4^+$ T cell proliferation. IL-23 is produced and secreted by activated dendritic cells, macrophages, B cells and γδ T cells. Excessive production of this cytokine can lead to autoimmune disorders, such as psoriasis, and even cancer. Ustekinumab (sold under the brand name STELARA®) and guselkumab (sold under the brand name Tremfya®) are two commercial monoclonal antibodies targeting IL-23, designed for the treatment of Crohn's disease, ulcerative colitis, plaque and arthritic psoriasis. The success of these two drugs indicates the relevance of this cytokine in human health and disease, making it a valuable therapeutic target.

Applications

Screening of small molecules and antibodies that inhibit binding of IL-23R to IL-23A.

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Supplied Materials

Catalog #	Name	Amount	Storage
	IL-23A[Biotin]*	2 µg	-80°C
	IL-23R*	20 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate		Room Temp

**The concentration of the protein is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

- 1x PBS (Phosphate-Buffered Saline, pH 7.4
- Sterile distilled water
- Luminometer or microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Rotating or rocker platform

Storage Conditions

This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be performed in duplicate.
- The assay should include “Blank”, “Positive Control”, “Ligand Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using Anti-hIL-23 antibody (R&D Systems #AF1716) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

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Step 1: Coat plate

1. Thaw **IL-23R** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
2. Dilute **IL-23R** to 4 ng/µl with 1x PBS (50 µl/ well, except "Ligand Control" wells).
3. Add 50 µl of **diluted IL-23R** to each well, except "Ligand Control" wells.
4. Add 50 µl of **PBS** to the "Ligand Control" wells.
5. Incubate at 4°C overnight.
6. Dilute 3-fold the **3x Immuno Buffer 1** with distilled water. This makes **1x Immuno Buffer 1**.

   Note: 30 ml of 1x Immuno Buffer 1 are enough for a 96-well plate. 3x Immuno Buffer 1 is necessary for other steps in the protocol.
7. Wash the plate three times using 100 µl of **1x Immuno Buffer 1** per well.
8. Tap the plate onto clean paper towel to remove the liquid.
9. Block the wells by adding 100 µl of **Blocking Buffer 2** to every well.
10. Incubate at Room Temperature (RT) for 1 hour.
11. Wash plate three times with 100 µl/well of **1x Immuno Buffer 1**.
12. Tap the plate onto clean paper towel to remove the liquid.

Step 2: Reaction

1. Prepare a **Master Mix** (25 µl/well): N wells x (10 µl of 3x Immuno Buffer 1 + 15 µl of distilled water).
2. Add 25 µl of **Master Mix** to every well.
3. Prepare the **Test Inhibitor** (5 µl/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

   3.1 If the Test Inhibitor is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration in 1x Immuno Buffer 1.

   For the positive and negative controls, use 1x Immuno Buffer 1 (Diluent Solution).

   OR

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3.2 If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 10-fold with 1x Immuno Buffer 1 (at this step the compound concentration is 10-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 10%.

Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in 1x Immuno Buffer 1 to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Immuno Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 µl of **Test Inhibitor** to each well labeled as "Test Inhibitor".

5. Add 5 µl of **Diluent Solution** to the "Positive Control", "Ligand Control" and "Blank" wells.

6. Thaw **IL-23A[Biotin]** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

7. Dilute **IL-23A** to 0.5 ng/µl with **1x Immuno Buffer 1** (20 µl/well).

8. Initiate the reaction by adding 20 µl of **diluted IL-23A** to the wells designated "Positive Control", "Ligand Control" and "Test Inhibitor."

9. Add 20 µl of **1x Immuno Buffer 1** to the "Blank" wells.

10. Incubate at RT for 2 hours.

	Blank	**Ligand Control (uncoated)**	**Positive Control**	**Test Inhibitor**
Master Mix	25 µl	25 µl	25 µl	25 µl
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
Diluted IL-23A (0.5 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

11. Wash the plate three times with 100 µl of **1x Immuno Buffer 1**. and tap the plate onto clean paper towel.

Step 3: Detection

1. Dilute **Streptavidin-HRP** 1000-fold in **Blocking Buffer 2** (100 µl/well).

2. Add 100 µl of **diluted Streptavidin-HRP** to each well.

3. Incubate for 1 hour at RT.

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4. Wash three times with 100 µl of **1x Immuno Buffer 1** and tap the plate onto clean paper towel.

5. Block the wells by adding 100 µl of **Blocking Buffer 2** to every well.

6. Incubate at RT for 10 minutes.

7. Tap the plate onto clean paper towel to remove the liquid.

8. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).

9. Add 100 µl of mix per well.

10. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.

11. The "Blank" value should be subtracted from all other values.

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 Results

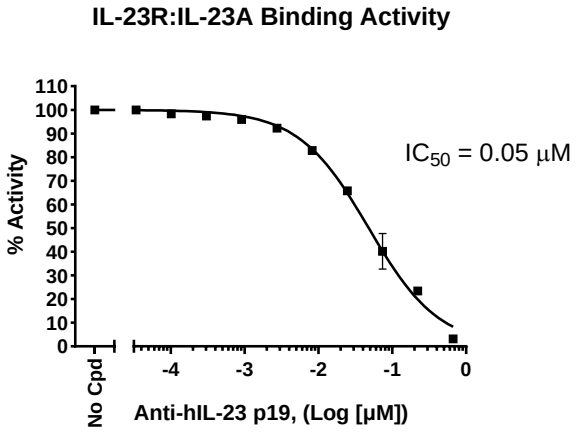

Figure 2: Inhibition of binding of IL-23R:IL-23A by anti-hIL-23 p19 antibody.
A plate was coated with IL-23R, followed by incubation with IL-23A in the presence of increasing concentrations of Human hIL-23 p19 Antibody (R&D Systems #AF1716). Luminescence was measured using a Bio-Tek microplate reader.

Data shown is representative.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit https://bpsbioscience.com/contact.

References
McKenzie B.S., *et al.* 2006 *Trends in Immunology* 27(1): 17-23.
Iwakura, Y. and Harumichi I. 2006 *The Journal of clinical investigation* 116(5): 1218-1222.
Fragoulis G. and Siebert S., 2022 *Musculoskeletal Care* 20 (suppl 1): S12-S21.

Related Products

Products	*Catalog #*	*Size*
IL-23α Neutralizing Antibody	101453	50 µg
IL23R, Fc-Fusion (IgG1), Avi-Tag Recombinant	101321	100 µg
IL23R, Fc-Fusion (IgG1), Avi-Tag, Biotin-Labeled Recombinant	101322	25 µg/100 µg
IL-23, His-Tag Recombinant	102096	5 µg/20 µg

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