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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The IL12B:IL12RB1 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is an ELISA designed for screening and profiling molecules that block the binding between IL12B (Interleukin 12B) and IL12RB1 (Interleukin 12 receptor subunit beta 1). This kit comes in a convenient 96-well format, with enough recombinant purified IL12B (amino acids 23-328(end)), biotin-labeled IL12RB1 (amino acids 24-540), streptavidin-labeled HRP, and assay buffer for 100 binding reactions.

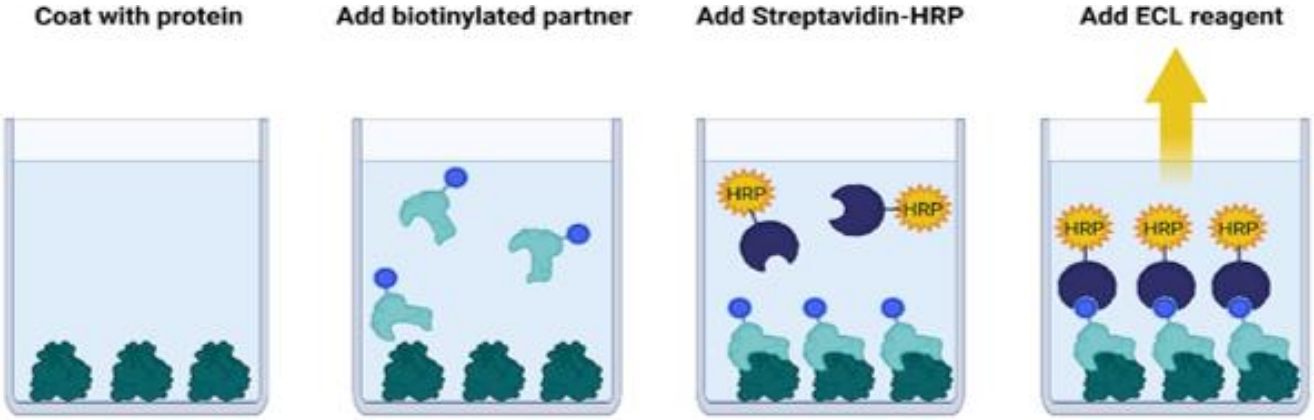

Figure 1: Illustration of the mechanism of IL12B: IL12RB1 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

A 96-well plate is coated with IL12B protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-IL12RB1, the plate is washed and Streptavidin-HRP is added. The ELISA ECL substrate is added, and the resulting signal can be measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of IL12B to IL12RB1.

Background

IL12B (Interleukin-12 subunit beta) encodes the p40 subunit of the heterodimeric cytokine IL12, which functions alongside the p35 subunit (encoded by IL12A). The IL12Rβ1 receptor subunit interacts with IL12B and plays a crucial role in the IL12 and IL23 signaling pathways, promoting Th1 cell differentiation and IFN-γ (Interferon-γ) production. Anti-p40 neutralizing antibodies, which target the p40 subunit common to both IL12 and IL23, are primarily used as therapeutic agents for autoimmune and inflammatory diseases. These antibodies have demonstrated significant efficacy in conditions such as psoriasis, psoriatic arthritis, and Crohn's disease by reducing inflammatory responses and modulating immune activity. The continued exploration of these antibodies holds promise for expanding their use to other immune-related conditions and improving patient outcomes.

Application(s)

Screen inhibitors or antibodies that block IL12B binding to IL12RB1.

Supplied Materials

Catalog #	Name	Amount	Storage
102210	IL12B, Avi-His-Tag*	50 µg	-80°C
	IL12RB1, Biotin-Labeled *	10 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 Substrates A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrates B (brown bottle)	6 ml	Room Temp
79699	White 96-well microplate	1	Room Temp

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

Materials Required but Not Supplied

1x PBS buffer (Phosphate Buffer Saline)
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

The DMSO concentration in the final reaction should be ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Non-Coated Condition”, “Blank”, “Positive Control” and “Test Inhibitor” wells.
- We recommend preincubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.

- We recommend using anti-p40 neutralizing antibody (#102108) as internal control. If not running a dose response curve, we recommend running the antibody at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).

Step 1 - Plate coating with IL12B protein

Coat the plate one day prior to running your samples in the assay.

1. Thaw **IL12B** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **IL12B** protein to 10 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **IL12B** protein solution to each well, except "Non-Coated Condition" wells.
4. Add 50 µl of PBS to "Non-Coated Condition" wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Immuno Buffer** by diluting 3-fold **3x Immuno Buffer** with distilled water.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
9. Tap the plate onto a clean paper towel to remove the liquid.
10. Add 100 µl of Blocking Buffer 2 to every well.
11. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
12. Tap the plate onto a clean paper towel to remove the liquid.
13. Start your assay immediately.

Step 2.1: Assessment of the inhibition/blocking of IL12B binding to IL12RB1 by an anti-p40 antibody or blocker.

1. Prepare a serial dilution of **anti-p40** antibody or blocker in Blocking Buffer 2 at the desired concentrations (50 µl/well).
2. Add 50 µl of the diluted antibody to the "Test Inhibitor" wells.
3. Add 100 µl of Blocking Buffer 2 to the "Blank" wells.
4. Add 50 µl of Blocking Buffer 2 to the "Positive Control" wells.

5. Incubate the plate for 30 minutes (up to 1 hour) at RT with gentle agitation.
6. Thaw the **IL12RB1-Biotin** on ice. Briefly spin the tube to recover the full content.
7. Dilute **IL12RB1-Biotin** to 2 µg/ml in Blocking Buffer 2 (50 µl/well).
8. Add 50 µl of diluted **IL12RB1-Biotin** to the "Test Inhibitor" and "Positive Control" wells.
9. Incubate the plate at RT for 1 hour with gentle agitation.
10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
11. Block the wells by adding 100 µl of Blocking Buffer 2 to every well and incubate for 10 minutes.
12. Tap the plate onto a clean paper towel to remove the liquid.

Step 3.1: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with the Blocking Buffer 2 (100 µl/well).
2. Add 100 µl of the diluted Streptavidin-HRP to each well.
3. Incubate the plate for 1 hour at RT with gentle agitation.
4. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.
5. Tap the plate onto a clean paper towel to remove the liquid.
6. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 µl of mix/well).
7. Add 100 µl of mix to each well.
    a. *Note: Discard any unused chemiluminescent mix after use.*
8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.
9. The "Blank" value should be subtracted from all readings.

	Blank	Positive Control	Test Inhibitor
Blocking Buffer 2	100 µl	50 µl	-
Test Inhibitor	-	-	50 µl
Diluted IL12RB1-Biotin (2 µg/ml)	-	50 µl	50 µl
Total	**100 µl**	**100 µl**	**100 µl**

Step 2.2: Assessment of the inhibition/blocking of IL12B binding to IL12RB1 by small molecules.

1. 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.

   1.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water at concentrations 10-fold higher than the desired final concentrations. Distilled water is the Diluent Solution.

   OR

   1.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using distilled water containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

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

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

2. Add 5 µl of diluted Test Inhibitor to each well labeled “Test Inhibitor”.

3. Add 5 µl of the Diluent Solution to the “Positive Control” and “Blank” wells.

4. Thaw **IL12RB1-Biotin** on ice. Briefly spin the tube to recover the full content.

5. Dilute IL12RB1-Biotin to 2 µg/ml in Blocking Buffer 2 (20 µl/well).

6. Add 20 µl of diluted IL12RB1-Biotin to the wells labeled “Test Inhibitor” and “Positive Control”.

7. Add 25 µl of Blocking Buffer 2 to the “Test Inhibitor” and “Positive Control” wells.

8. Add 45 µl of Blocking Buffer 2 to the “Blank” wells.

9. Incubate the plate at RT for 1 hour with gentle agitation.

10. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.

11. Block the wells by adding 100µl of Blocking Buffer 2 to every well and incubate for 10 minutes.

12. Tap the plate onto a clean paper towel to remove the liquid.

	Blank	Positive Control	Test Inhibitor
Blocking Buffer 2	45 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted IL12RB1-Biotin (2 µg/ml)	-	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**

Step 3.2: Detection

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

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

3. Incubate the plate for 1 hour at RT with gentle agitation.

4. Wash the plate three times with 100 µl of 1x Immuno Buffer 1 per well.

5. Tap the plate onto a clean paper towel to remove the liquid.

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

7. Add 100 µl of mix to each well.
    a. *Note: Discard any unused chemiluminescent mix after use.*

8. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

9. The "Blank" value should be 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 enzyme (typically we set this value as 100).

Example Results

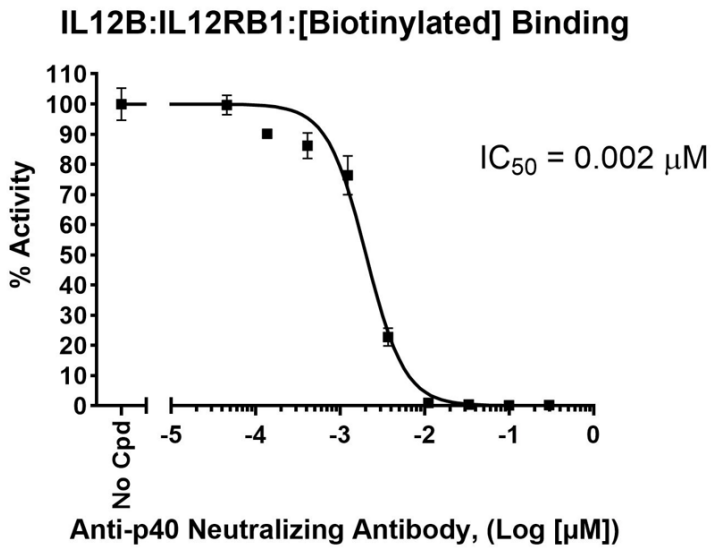

Figure 1. Inhibition of IL12B:IL12RB1 binding by Anti-p40 Neutralizing Antibody.
IL12B:IL12RB1 binding was evaluated in the presence of increasing concentrations of Anti-p40 Neutralizing Antibody (#102108). Results are expressed as percent activity, in which the binding activity in the absence of inhibitor is set to 100%.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide
Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

Related Products

Products	*Catalog #*	*Size*
Anti-p40 Neutralizing Antibody	102108	25 µg/100 µg/1 mg
IL-12 (p40/p35) Fc Fusion (IgG1), Avi-Tag, Biotin-Labeled Recombinant	101432	25 µg/100 µg
IL-12 (p40/p35) Fc Fusion (IgG1), Avi-Tag Recombinant	101431	100 µg
IL12RB1, Avi-His-Tag Recombinant	101336	100 µg

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