CD125 (IL-5RA): IL-5 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit

#82965
96 reactions

Description

The CD125 (IL-5RA): IL-5 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is an ELISA-based assay designed to measure the binding between CD125 (also known as IL-5RA or Interleukin 5 receptor subunit alpha) and IL-5 protein for screening and profiling applications. This kit comes with enough purified CD125 (amino acids 21-342) and biotinylated IL-5 (amino acids 20-134(end)), streptavidin-HRP, assay buffer, and detection reagent for 100 enzyme reactions.

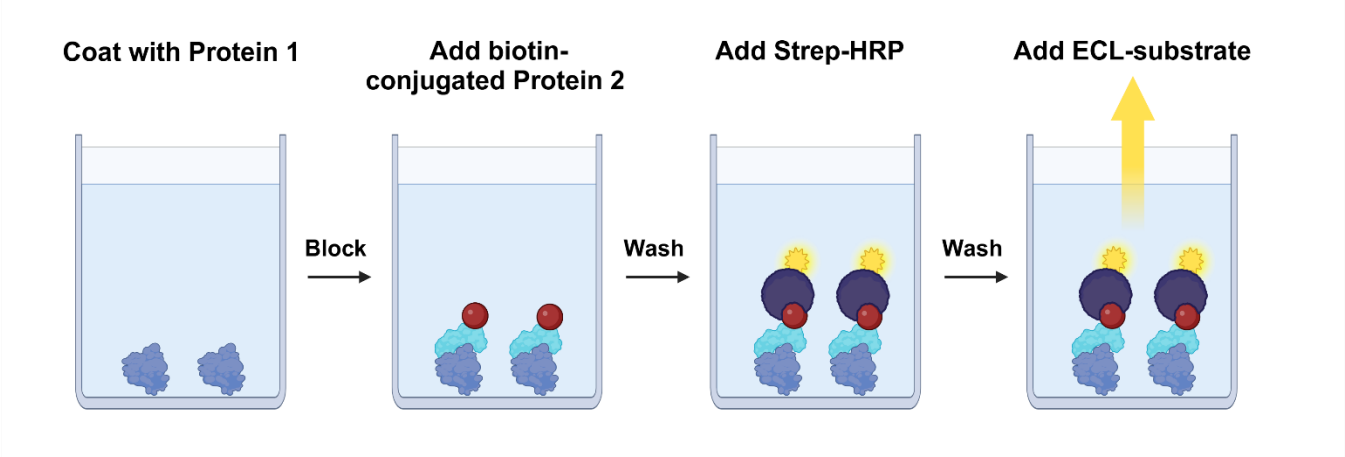

Figure 1. CD125 (IL-5RA): IL-5 [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit schematic.
A 96-well plate is coated with CD125 protein. After coating and blocking, biotinylated IL-5 is added in an optimized assay buffer. Next, unbound biotinylated IL-5 is washed away, and the plate is 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-5 binding to CD125.

Background

IL-5 (interleukin-5) is a pro-inflammatory protein that functions as an intermediary cytokine in the TH2 (T helper 2) cells response in asthma and other eosinophilic allergic diseases. IL-5 functions by promoting eosinophil maturation, activation, and migration into target tissues. It binds to the IL-5 specific cell surface receptor IL-5RA (IL-5 receptor alpha) and further recruits the common beta chain receptor (CSF2RB) to initiate downstream signaling through JAK1/2 (Janus kinase 1/2) and STAT5. Activated eosinophils contribute to airway damage through degranulation, resulting in the release of pro-inflammatory cytokines and enzymatic mediators contributing to local inflammation and tissue damage. IL-5 signaling plays a critical role in allergic and eosinophilic asthma subtypes and has been an active area for drug discovery. Antibodies targeting both IL-5 ligand and CD125 have been FDA (Food and Drug Administration) approved for the treatment of severe eosinophilic asthma and other eosinophilic conditions. These antibodies function by neutralizing IL-5-mediated signaling in eosinophils. Additionally, IL-5Ra targeting antibodies and CAR (chimeric antigen receptor)-T cells can induce immune-mediated cytotoxicity of IL-5RA-expressing cells, including eosinophils through ADCC (antibody-dependent cellular cytotoxicity) and CAR-T cell mediated killing respectively.

Applications

Study and screen compounds that inhibit the binding of CD125 to IL-5 for drug discovery in high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
102645	CD125 (IL-5Ra), Fc-Fusion (lgG1), Avi-Tag*	10 µg	-80°C
102507	IL-5, His-Avi-Tag, Biotin-Labeled*	10 µg	-80°C
82620	5x PP-02 Buffer	4 ml	-20°C
82765	Blocking Buffer 9	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	1	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 Buffer Saline) Buffer
- PBST Buffer (1x PBS, containing 0.05% Tween-20)
- 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 CD125 (IL-5RA): IL-5 Binding Assay Kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples should be run in duplicate while controls should be performed in quadruplicate.
- The assay should include “Blank”, “Positive 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-IL-5RA Antibody (#102485) 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.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

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Step 1: Coat 96-well plate
Coat the plate one day prior to running your samples.

1. Thaw **CD125** on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute CD125 protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted CD125 to every well, except "Blank" wells.
4. Add 50 µl of **Blocking Buffer 9** to the "Blank" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times using 200 µl of PBST Buffer per well.
7. Tap the plate onto a clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 9 to every well.
9. Incubate at Room Temperature (RT) for at least 90 minutes.
10. Wash the plate three times using 200 µl of PBST Buffer per well.
11. Tap the plate onto a clean paper towel to remove the liquid.

Step 2: Binding reaction

1. Prepare **1x Assay Buffer** by diluting **5x PP-02 Assay Buffer** 5-fold with distilled water.
2. Add 20 µl of 1x Assay Buffer to every well.
3. Prepare the **Test Inhibitor/Blocker** (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/Blocker is soluble in water, prepare a solution of the compound that is 10-fold higher than the final desired concentration using 1x Assay Buffer.

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

   OR

   3.2 If the Test Inhibitor/Blocker 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

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1x Assay Buffer (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 Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Assay Buffer (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" and "Blank" wells.
6. Thaw **IL-5** on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute IL-5 to 4 ng/µl with 1x Assay Buffer (25 µl/well).
8. Add 25 µl of diluted IL-5 to all wells.
9. Incubate at RT for 1 hour.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	20 µl	20 µl	20 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted IL-5 (4 ng/µl)	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**

10. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

Step 3: Detection

1. Dilute 1000-fold the **Streptavidin-HRP** with Blocking Buffer 9 (50 µl/well).
2. Add 50 µl of diluted Streptavidin-HRP to every well.
3. Incubate for 1 hour at RT.
4. Wash the plate three times with 200 µl of PBST Buffer per well and tap the plate onto clean paper towel.

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

6. Add 100 µl of mix to every well.

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

8. 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 controls.

Example Results

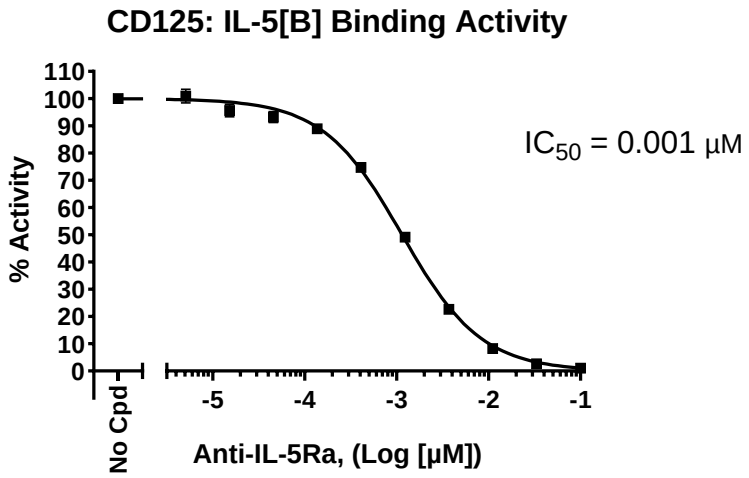

Figure 2: Inhibition of CD125-IL-5 binding by Anti-IL-5RA Antibody.
IL-5 was incubated with increasing concentrations of Anti-IL-5RA Antibody (#102485) in a CD125 coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without Anti-IL-5RA Antibody is set to 100%.

Data shown is representative.

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References

Schleich F*., et al.,* 2023 *Eur Respir Rev* 32(168): 220193.

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.

Related Products

Products	*Catalog #*	*Size*
IL-5: CD125 (IL-5RA) [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	82964	96 reactions
IL-5 Responsive Luciferase Reporter Ba/F3 Cell Line	82731	2 vials
IL-5, His-Avi-Tag, Biotin-Labeled Recombinant	102507	25 µg/ 100 µg/ 1 mg
IL-23R: IL-23A [Biotinylated] Inhibitor Screening Assay Kit	78014	96 reactions

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