Description

The IL1RL1: IL33 [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit is an ELISA-based assay designed to measure the binding between IL1RL1 (interleukin 1 receptor like 1 or IL33R) and IL-33 protein for screening and profiling applications. This kit comes with enough purified IL1RL1 (amino acids 19-328) and biotinylated IL33 (amino acids 109-270), streptavidin-HRP, assay buffer, and detection reagent for 100 enzyme reactions.

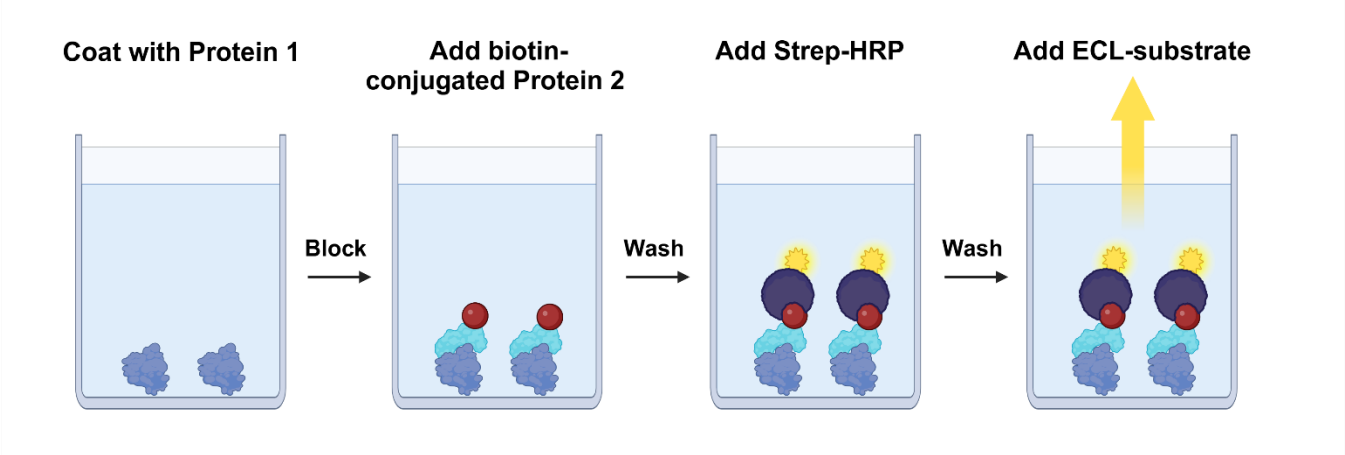

Figure 1. IL1RL1: IL33 [Biotinylated] Inhibitor Screening Chemiluminescent Assay Kit schematic.
A 96-well plate is coated with IL1RL1 protein. After coating and blocking, biotinylated IL33 is added in an optimized assay buffer. Next, unbound biotinylated IL33 is washed away, and the plate is incubated 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 IL33 binding to IL1RL1.

Background

IL33 is a cytokine of the IL1 family, that is released in response to external triggers, such as trauma, allergen exposure and infections. IL33 can be either on its reduced or oxidized state, which use different signaling pathways. When in its reduced state ($IL33^{red}$) it binds to IL1RL1 (interleukin 1 receptor like 1), also named IL33R or ST2 (receptor serum-stimulated 2). IL1RL1 exists as both a membrane bound protein or a truncated soluble form. Binding of IL33 to IL1RL1 initiated a signaling pathway that involves NF-κF (nuclear factor kappa-light-chain-enhancer of activated B cells) and MAPK (mitogen-activated protein kinase), and results in release of pro-inflammatory cytokine and chemokines. The soluble form of IL1RL1 can act as a decoy receptor for IL33. Dysregulation in the levels of IL33 is implicated in the pathology of inflammatory and infectious diseases, including COVID-19, COPD (chronic obstructive pulmonary disease) and asthma. The involvement of IL33 in these diseases has made it a high value therapeutic target, with strategies such as antibodies being developed to inhibit the binding of IL33 to its receptor. One example is tozorakimab (MEDI3506), from AstraZeneca, currently being evaluated as treatment for COPD. Further studies on IL33 and its receptor will likely result in new therapies for IL33 related disorders.

Applications

Study and screen compounds that inhibit the binding of IL1RL1 to IL33 for drug discovery in high throughput screening (HTS) applications.

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Supplied Materials

Catalog #	Name	Amount	Storage
102451	IL1RL1 (IL33R) Fc-tag, Avi-tag*	10 µg	-80°C
102421	IL33, His-tag, Avi-tag, Biotin Labeled*	7.5 µg	-80°C
82620	5x PP-02 Buffer	4 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	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-Buffered Saline, pH 7.4)
- 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

This assay 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”, 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-IL33 Neutralizing Antibody (#102416) 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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- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

Step 1: Coat 96-well plate

Coat the plate one day prior to running your samples.

1. Thaw IL1RL1 on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute IL1RL1 protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted IL1RL1 to every well, except "Blank" wells.
4. Add 100 µl of Blocking Buffer 2 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 clean paper towel to remove the liquid.
8. Block the wells by adding 200 µl of Blocking Buffer 2 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 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 IL33 on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute IL33 to 3 ng/µl with 1x Assay Buffer (25 µl/well).
8. Add 25 µl of diluted IL33 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 IL33 (3 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 2 (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

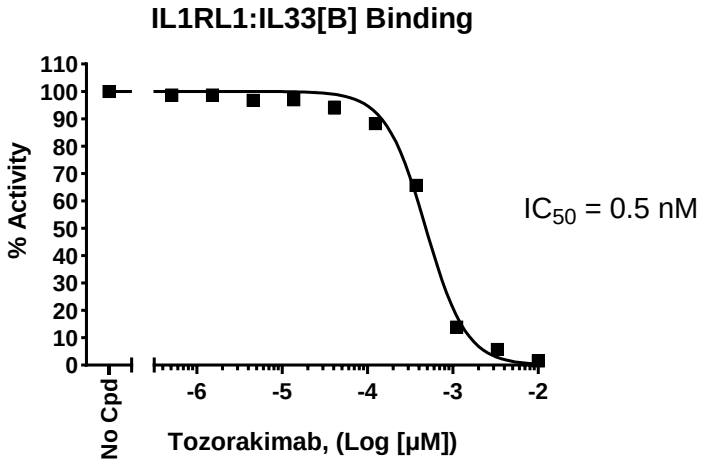

Figure 2: Inhibition of IL33 binding to IL1RL1 by Anti-IL33 Antibody.
IL33 was incubated with increasing concentrations of Anti-IL33 Neutralizing Antibody (#102416) in an IL1RL1 coated plate. Luminescence was measured using a Bio-Tek microplate reader. Results are expressed as a percentage of binding activity in which the condition without antibody is set to 100%.

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

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References

England E., *et al.*, 2023 *Scientific Reports* 13:9825.

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*
Human Interleukin-33 Recombinant	90191	2 µg/ 10 µg
Human Interleukin-1 receptor antagonist Recombinant	90170	20 µg/ 100 µg
IL-15 Responsive Luciferase Reporter Cell Line	78402	2 vials
Human Interleukin-15 Recombinant	90180	2 µg/ 10 µg
IL12B:IL12RB1[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	82581	96 reactions

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