Description

The IL-17RA[Biotinylated]:IL-17A Inhibitor Screening Assay Kit is designed to measure the binding of IL-17RA (interleukin 17 receptor A) to IL-17A (IL-17 subunit alpha) for screening and profiling applications. The IL-17RA[Biotinylated]:IL-17A Inhibitor Screening Assay Kit comes in a convenient 96-well format, with enough recombinant purified biotinylated IL-17RA (amino acids 33-320), IL-17A (amino acids 20-155), blocking and assay buffer and detection reagents for 100 enzyme reactions.

Background

IL-17 (interleukin 17) is a cytokine involved in inflammation and belongs to the pro-inflammatory cystine knot cytokine family. The IL-17 family includes IL-17A-F. It binds to the receptor IL-17R, which has three variants IL-17RA-C, in T helper 17 (Th17) cells in response to IL-23. It is also produced by macrophages, dendritic cells and δγ T cells. Activation of downstream pathways leads to the release of chemokines, which can recruit immune cells to inflammation sites, cytokines (such as IL-6 and GCSF (granulocyte colony stimulating factor)) and complement proteins. IL-17A is also involved in differentiation of $CD34^+$ hematopoietic progenitor cells into neutrophils. Higher levels IL-17A have been linked to autoimmune disorders such as RA (rheumatoid arthritis), lupus psoriasis and asthma, transplant rejection and MS (multiple sclerosis). Inhibitors of IL-17 have been under development, as treatment options for autoimmune diseases, with the first monoclonal antibody being approved by the FDA in 2015 for the treatment of plaque psoriasis (secukinumab, sold under the commercial name of Cosentyx). The success of these 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-17RA to IL-17A.

Supplied Materials

Catalog #	Name	Amount	Storage
91013	IL-17RA, Fc Fusion, Biotin-Labeled (Human)*	2 µg	-80°C
91014	IL-17A, Avi-Tag*	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 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 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

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-IL-17A Neutralizing Antibody (BPS Bioscience #91015) 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.

Step 1: Coat plate

1. Thaw **IL-17A** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.
2. Dilute IL-17A to 2 µg/ml with 1x PBS (50 µl/well, except "Ligand Control" wells).
3. Add 50 µl of diluted IL-17A to each well, except "Ligand Control" wells.
4. Incubate at 4°C overnight.
5. 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.
6. Wash the plate three times using 100 µl of 1x Immuno Buffer 1 per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.
9. Incubate at Room Temperature (RT) for 1 hour.
10. 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 distilled water.

   For the positive and negative controls, use distilled water (Diluent Solution).

   OR

   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 distilled water (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 distilled water to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 10% DMSO in distilled water 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-17RA** enzyme on ice. Briefly spin the tube containing the enzyme to recover its full content.

7. Dilute IL-17RA to **0.5 ng/µl** with 1x Immuno Buffer 1 (20 µl/well).

8. Initiate the reaction by adding 20 µl of diluted IL-17RA 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-17RA (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.
12. Block the wells by adding 100 µl of Blocking Buffer 2 to every well.
13. Incubate at RT for 10 minutes.
14. Tap the plate onto clean paper towel to remove the liquid.

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.
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).

Example Results

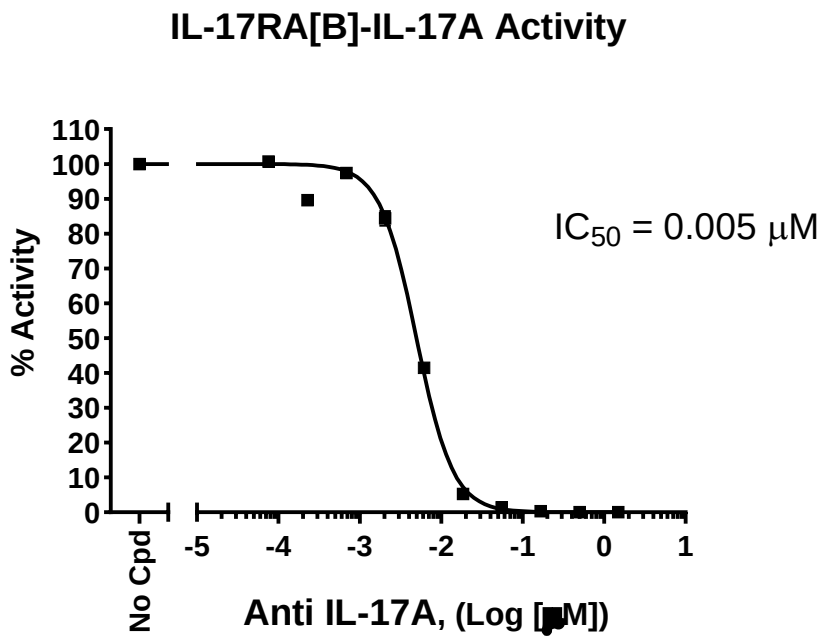

Figure 1: Inhibition of binding of IL-17RA:IL-17A by Anti-IL-17A Neutralizing Antibody.
A plate was coated with IL-17A, followed by incubation with IL-17RA in the presence of increasing concentrations of Anti-IL-17 Neutralizing Antibody (#91015). Luminescence was measured using a Bio-Tek microplate reader.

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

References

Iwakura Y., *et al.* 2008 *Immunol Rev.* 226:57-79.
Bullens D.M., *et al.* 2013 *Clin Dev Immunol.* 2013:840315.
Ley K., *et al.* 2006 *Immunol Res.* 34(3):229-42.
Tiburca L., *et al.*, 2022 *Curr Issues Mol Biol* 44(5):1851-1866.

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-IL-17A Neutralizing Antibody	91015	100 µg
IL-17RA:IL-17A[Biotinylated] Inhibitor Screening Assay Kit	79891	96 reactions
IL-17RA, Fc-Fusion, Avi-Tag Recombinant	11264	100 µg
NF-κB Reporter (Luc) – HEK293 Recombinant Cell Line	60650	2 vials

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