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Lieferung & Zahlungsart

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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The IL-17RA [Biotinylated]:IL-17A: Inhibitor Screening Assay Kit is an ELISA 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.

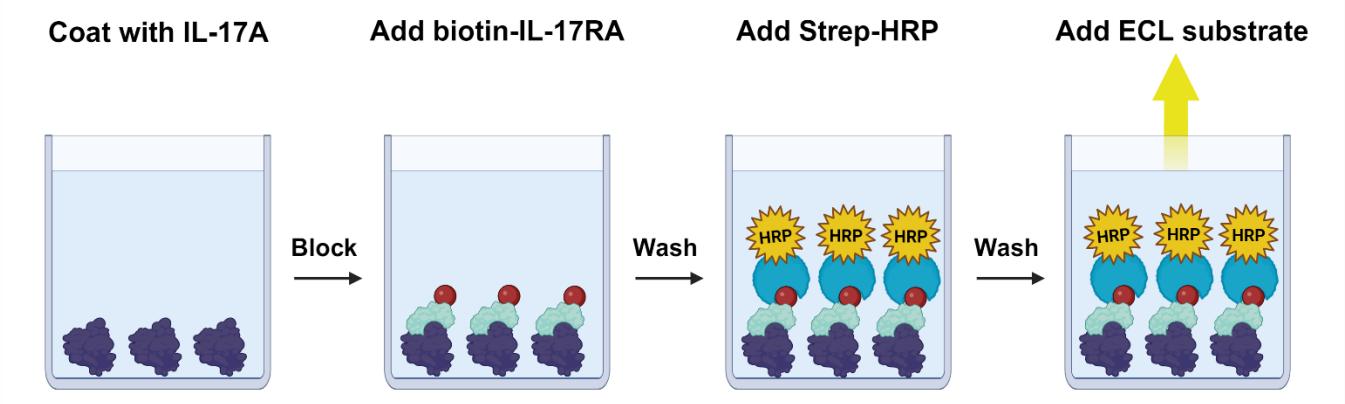

Figure 1. IL-17RA [Biotinylated]: IL-17A Inhibitor Screening Assay Kit schematic.
A 96-well plate is coated with IL-17A protein. After blocking, biotinylated IL-17RA is added in an optimized assay buffer. Unbound biotinylated IL-17RA is washed away, and the plate is incubated with streptavidin-HRP. After 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-17A binding to IL-17RA.

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 to F. It binds to the receptor IL-17R, which has three variants including IL-17RA-C in T helper 17 (Th17) cells, which also responds to IL-23. It is 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 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

Study and screen compounds that inhibit the binding of IL-17RA to IL-17A for drug discovery in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
91014	IL-17A, Avi-Tag*	10 µg	-80°C
91013	IL-17RA, Fc Fusion, Biotin-Labeled (Human)*	1 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
	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
79670	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

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”, 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 (#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.

- 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 IL-17A on ice. Briefly spin the tube containing the protein to recover its full content.
2. Dilute IL-17A protein to 2 ng/µl with 1x PBS (50 µl/well).
3. Add 50 µl of diluted IL-17A to every well, except "Blank" wells.
4. Add 200 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 towels 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 towels to remove the liquid.

Step 2: Binding reaction

1. Prepare 1x Assay Buffer by diluting 3x Immuno Buffer 3-fold with distilled water.
2. Add 25 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

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-17RA[B] on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute IL-17RA[B] to 0.5 ng/µl with 1x Assay Buffer (20 µl/well).
8. Add 20 µl of diluted IL-17RA[B] to all wells.
9. Incubate at RT for 2 hours.

	Blank (non-coated wells)	**Positive Control**	**Test Inhibitor**
1x Assay Buffer	25 µl	25 µl	25 µl
Test Inhibitor	-	-	5 µl
Diluent Solution	5 µl	5 µl	-
Diluted IL-17RA[B] (0.5 ng/µl)	20 µl	20 µl	20 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 towels.
11. Block by adding 200 µl of Blocking Buffer 2 to every well for 10 min.
12. Tap the plate onto clean paper towels.

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 towels.

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

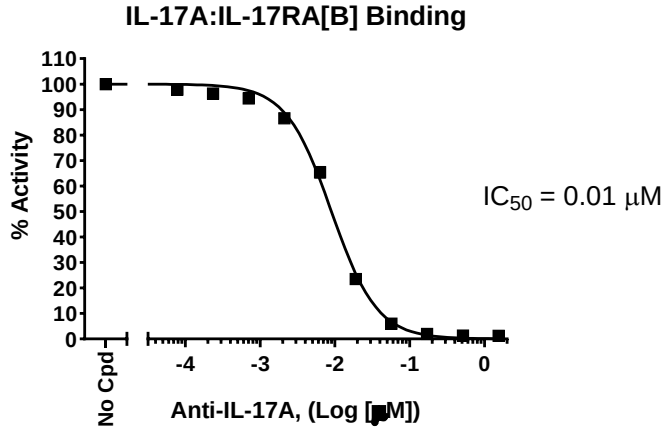

Figure 2: Inhibition of IL-17RA[B]:IL-17A binding by Anti-IL-17A Neutralizing Antibody.
IL-17RA was incubated with increasing concentrations of Anti-IL-17A Neutralizing Antibody (#91015) in an IL-17A coated plate. Luminescence was measured using a Bio-Tek microplate reader.

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/ 384 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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