

Description

The TSLPR:TSLP [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling molecules that block the binding between TSLPR (thymic stromal lymphopoietin receptor) and TSLP. This kit comes in a convenient 96-well format, with enough recombinant human biotin-labeled TSLP (amino acids 29-159), human TSLPR (amino acids 25-231), streptavidin-HRP, and assay buffer for 100 reactions.

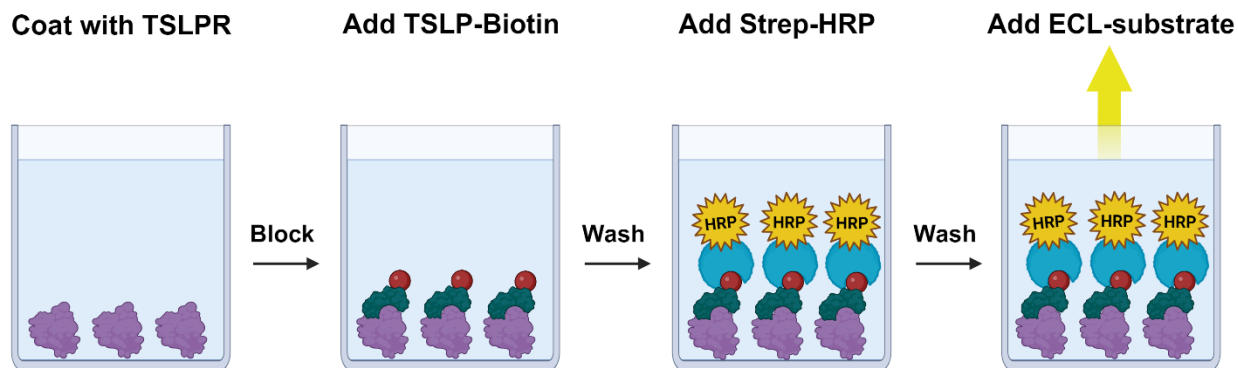


Figure 1: Illustration of the mechanism of TSLPR: TSLP [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

A 96-well plate is coated with TSLPR protein. After coating and blocking, TSLP is added in an optimized assay buffer. Unbound TSLP is washed away, and the plate is incubated with streptavidin-HRP. Finally, 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 TSLP binding to TSLPR.

Background

TSLP (thymic stromal lymphopoietin) is a type I cytokine that functions as an alarmin and growth factor in the immune system. It is involved in type 2 immune responses, T_H2 (T helper 2 cells) responses, and the maturation and recruitment of dendritic cells (DCs), T cells, B cells, neutrophils, mast cells, and other lymphoid cells. It can be produced by epithelial and stromal cells in the lung, skin, and gastric systems, but also by DCs, basophils and mast cells. Its expression can be induced by infections, pro-inflammatory cytokines, proteases, and even mechanical injury. For instance, it can be produced in the lungs in response to infection with influenza or rhinovirus. Its role as an alarmin can result in increasing inflammation. TSLP is linked to allergic reactions such as asthma, atopic dermatitis, and food allergies, by inducing the expression of OX40L, CD80 and CD86 and stimulating $CD4^+$ T cells. In 2021, the TSLP-neutralizing antibody tezepelumab was approved for the treatment of severe asthma. Targeting TSLP is an active area of investigation with ongoing clinical trials for the treatment of autoimmune disorders.

Application(s)

Screen small molecule inhibitors that block TSLP binding to TSLPR.

Supplied Materials

Catalog #	Name	Amount	Storage
102169	TSLP, Avi-Tag, His-Tag, Biotin-Labeled*	>1 µg	-80°C
102163	TSLPR, Fc-Tag*	20 µg	-80°C
82620	5x PP-02 Buffer	4 ml	-20°C
79743	Blocking Buffer 3	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 Buffered Saline, pH 7.4)
- PBST (1x PBS buffer with 0.05% Tween-20)
- 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 ≤3%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control” and “Test Compound” wells.
- For small molecule inhibitors, pre-incubation may be beneficial, depending on the experimental conditions.
- Baicalein (Cayman # 70610) may be used as internal control. If not running a dose response curve, we recommend running the control inhibitor at 0.5X, 1X and 2X the IC₅₀ 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 \(bpsbioscience.com\)](https://www.bpsbioscience.com).
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](https://www.bpsbioscience.com).

Step 1 - Plate coating with TSLPR

Coat the plate one day prior to running your samples.

1. Thaw **TSLPR** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **TSLPR** protein to 4 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **TSLPR** protein solution to each well, except “Blank” wells.
4. Add 50 µl of Blocking Buffer 3 to “Blank” wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times with 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 3 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 Compound (10 µl/well): for a titration, prepare serial dilutions at concentrations 5-fold higher than the desired final concentrations. The final volume of the reaction is 50 µl.

Note: Assay performance was verified with up to 3% DMSO. Thus, the DMSO concentration in the final reaction should be ≤3%.

- 3.1 If the Test Compound is soluble in water, prepare a solution of the compound that is 5-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 Compound is dissolved in DMSO and your desired final concentration of DMSO in the reaction is 1%, prepare a solution of the compound in 100% DMSO that is 100-fold higher than the highest concentration of the serial dilution. Then dilute 20-fold with 1x Assay Buffer (at this step the compound concentration is 5-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 5%.

Prepare serial dilutions of the Test Compound at concentrations 5-fold higher than the desired final concentrations using 5% DMSO in 1x Assay Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 5% DMSO in 1x Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

4. Add 10 µl of diluted Test Compound to each well labeled as "Test Compound".
5. Add 10 µl of Diluent Solution to the "Positive Control" and "Blank" wells.
6. Thaw **TSLP-Biotin** on ice. Briefly spin the tube containing the protein to recover its full content.
7. Dilute **TSLP-Biotin** to 0.4 µg/ml with 1x Assay Buffer (20 µl/well).
8. Add 20 µl of diluted TSLP-Biotin to all wells.
9. Incubate at RT for 2 hours with gentle agitation.

Note: If needed, reaction time can be extended to an overnight (18h) incubation at 4 °C.

	Blank (non-coated wells)	Positive Control	Test Compound
1x Assay Buffer	20 µl	20 µl	20 µl
Diluent Solution	10 µl	10 µl	-
Test Compound	-	-	10 µl
Diluted TSLP-Biotin (0.4 µg/ml)	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 a clean paper towel.

Step 3 Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 3 (50 µl/well).
2. Add 50 µl of 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 200 µl of PBST Buffer per well and tap the plate onto a clean paper towel.
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 each well.

Note: Discard any unused chemiluminescent mix after use.

7. Immediately read the plate in a luminometer or 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 a control assay without enzyme (typically we set this value as 100).

Example Results

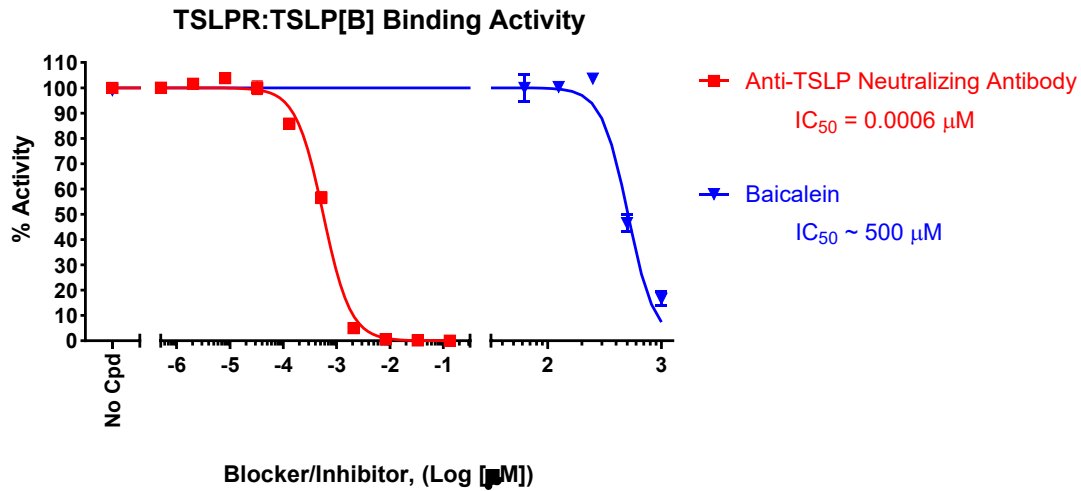


Figure 2. Inhibition of TSLP to TSLPR binding by Anti-TSLP Neutralizing Antibody and Baicalein. TSLPR:TSLP binding was evaluated in the presence of increasing concentrations of Anti-TSLP Neutralizing Antibody (#102138) or Baicalein (Cayman #70610). Results are expressed as percent activity, in which binding activity in the absence of inhibitor/blocker is set to 100%.

Data shown is representative.

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
TSLPR: TSLP[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit	82507	96 reactions
Anti-TSLP Neutralizing Antibody	102138	25 µg/100 µg
TSLP Responsive Luciferase Reporter Ba/F3 Cell Line	82500	2 vials
TSLP Responsive Luciferase Reporter U937 Cell Line	82501	2 vials
TSLP Avi-Tag, His-Tag Recombinant	102168	25 µg/100 µg/500 µg/1 mg
Human Thymic stromal lymphopoietin Recombinant	90250	2 µg/ 10 µg

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