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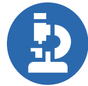
Produktinformation

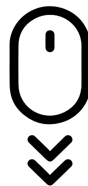
Forschungsprodukte & Biochemikalien

Zellkultur & Verbrauchsmaterial

Diagnostik & molekulare Diagnostik

 Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!



Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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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 TSLP-R (amino acids 25-231), streptavidin-HRP, and assay buffer for 100 reactions.

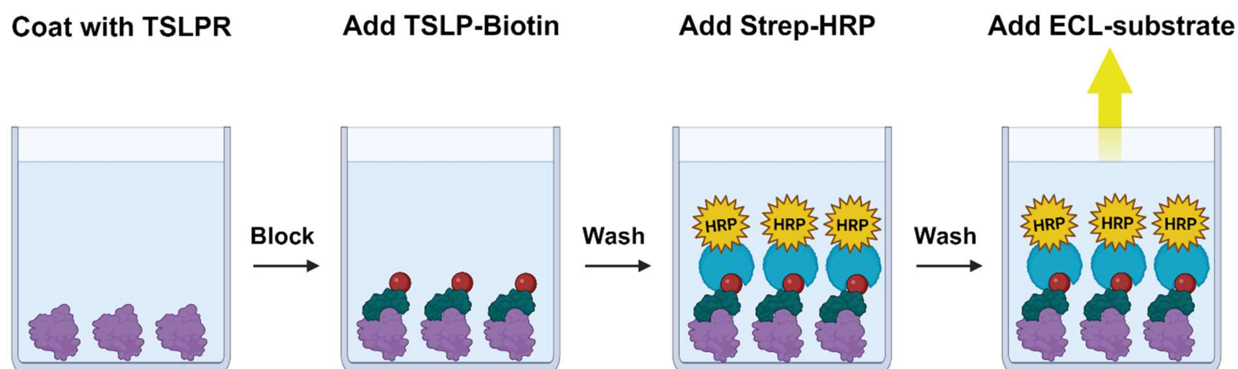

Figure 1: Illustration of the mechanism of TSLPR: TSLP [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.

A 96-well plate is coated with TSLPR protein. After blocking, the plate is pre-incubated with an inhibitor or neutralizing antibody. After incubation with Biotin-TSLP, the plate is washed and Streptavidin-HRP is added. The ELISA ECL substrate is added and the resulting signal can be measured using a chemiluminescence microplate reader. The chemiluminescence signal is proportional to the binding of TSLPR to TSLP.

Background

TSLP (thymic stromal lymphopoietin) is a protein that functions as a type I cytokine, 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 lung, skin, and gastric system, 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 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 TLSP-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 or antibodies 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
	5x PP-02 Buffer	4 ml	-20°C
	Blocking Buffer 7	40 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 both TSLP-R and TSLP is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

- 1x PBS buffer (Phosphate Buffer Saline, pH 7.4)
- PBS-T (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 ≤1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include "Non-Coated Control", "Blank", "Positive Control" and "Test Compound" wells.
- We recommend pre-incubating antibodies or protein inhibitors with the target protein prior to the addition of the binding partner.
- For small molecule inhibitors, pre-incubation may also be beneficial, depending on the experimental conditions.
- Anti-TSLP Neutralizing Antibody (#102138) may be used as internal control. If not running a dose response curve, we recommend running the antibody at 0.1X, 1X and 10X the IC_{50} 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).
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (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.
4. Add 50 µl of PBS to the "Non-Coated Control" wells.
5. Incubate at 4°C overnight.
6. Wash the plate three times with 200 µl of PBS-T Buffer per well.
7. Tap the plate onto clean paper towel to remove the liquid.
8. Add 100 µl of Blocking Buffer 7 to every well.
9. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
10. Tap the plate onto clean paper towel to remove the liquid.
11. Start your testing immediately.

Step 2.1: Assessment of the inhibition/blocking of TSLP binding to TSLPR by anti-TSLPR or anti-TSLP antibodies.

1. Prepare **1x PP-02 Buffer** by diluting 5-fold the **5x PP-02 Buffer** with distilled water.
2. Prepare a serial dilution of **anti-TSLP or anti-TSLPR** antibody or blocker of interest in 1x PP-02 Buffer at 5X the final desired concentrations (10 µl/well).

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3. Add 10 µl of the diluted antibody/blocker to the “Test Compound” wells.

4. Add 20 µl of 1x PP-02 Buffer to the “Test Compound” wells.

5. Add 50 µl of 1x PP-02 Buffer to the “Blank” wells.

6. Add 30 µl of 1x PP-02 Buffer to the “Positive Control” and “Non-Coated Control” wells.

7. Incubate the plate for 30 minutes at RT with gentle agitation.

8. Thaw the **TSLP-Biotin** on ice. Briefly spin the tube to recover the full content.

9. Dilute **TSLP-Biotin** to 0.4 µg/ml with 1x PP-02 Buffer (20 µl/well).

10. Add 20 µl of diluted **TSLP-Biotin** to the “Non-Coated Control, “Test Compound”, and “Positive Control” wells.

11. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Ligand Control**	**Positive Control**	**Test Compound**
PP-02 Buffer	50 µl	30 µl	30 µl	20 µl
Test Compound	-	-	-	10 µl
Pre-incubate 30 minutes at RT				
Diluted TSLP-Biotin (0.4 µg/ml)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

12. Wash the plate three times with 200 µl of PBS-T Buffer per well.

13. Tap the plate onto clean paper towel to remove the liquid.

14. Block by adding 100 µl of Blocking Buffer 7 to every well and incubate for 10 minutes at RT.

15. Tap the plate onto clean paper towel to remove the liquid.

Step 3.1: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 7 (50 µl/well).

2. Add 50 µl of diluted **Streptavidin-HRP** to each well.

3. Incubate the plate for 30 minutes at RT with gentle agitation.

4. Wash the plate three times with 200 µl of PBS-T Buffer per well.

5. Block the wells by adding 100 µl of Blocking Buffer 7 to every well and incubate for 10 minutes at RT.

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6. Tap the plate onto clean paper towel to remove the liquid.

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

8. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.

9. Immediately read the plate in a luminometer or plate reader capable of reading chemiluminescence.

10. The "Blank" value should be subtracted from all readings.

Step 2.2: Assessment of the inhibition/blocking of TSLP binding to TSLPR by small molecules.

1. 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.

   1.1 If the Test Inhibitor is water-soluble, prepare serial dilutions in distilled water at concentrations 10-fold higher than the desired final concentrations.

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

   OR

   1.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor in 100% DMSO at a concentration 100-fold higher than the highest desired final concentration, then dilute the inhibitor 10-fold in distilled water to prepare the highest concentration of the serial dilutions. The concentration of DMSO is now 10%.

   Using distilled water containing 10% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 10% DMSO in distilled water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO in the assay should not exceed 1%.

2. Add 5 µl of diluted Test Inhibitor to each well labeled "Test Compound".

3. Add 5 µl of the Diluent Solution to the ""Non-Coated Control", "Positive Control", and "Blank" wells.

4. Prepare **1x PP-02 Buffer** by diluting 5-fold the **5x PP-02 Buffer** with distilled water.

5. Thaw **TSLP-Biotin** on ice. Briefly spin the tube to recover the full content.

6. Dilute **TSLP-Biotin** to 0.4 µg/ml with 1x PP-02 Buffer (20 µl/well).

7. Add 20 µl of diluted **TSLP-Biotin** to the wells labeled "Non-Coated Control", "Test Compound", and "Positive Control".

8. Add 25 µl of 1x PP-02 Buffer to the "Non-Coated Control", "Test Compound", and "Positive Control" wells.

9. Add 45 µl of PP-02 Buffer to the "Blank" wells.

10. Incubate the plate at RT for 1 hour with gentle agitation.

	Blank	**Ligand Control**	**Positive Control**	**Test Compound**
Test Inhibitor	-	-	-	5 µl
Diluent Solution	5 µl	5 µl	5 µl	-
Diluted TSLP-Biotin (0.4 µg/ml)	-	20 µl	20 µl	20 µl
1x PP-02 Buffer	45 µl	25 µl	25 µl	25 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

11. Wash the plate three times with 200 µl of PBS-T Buffer per well.

12. Tap the plate onto clean paper towel to remove the liquid.

13. Block by adding 100 µl of Blocking Buffer 7 to every well and incubate for 10 minutes at RT.

14. Tap the plate onto clean paper towel to remove the liquid.

Step 3.2: Detection

1. Dilute **Streptavidin-HRP** 1000-fold with Blocking Buffer 7 (50 µl/well).

2. Add 50 µl of diluted Streptavidin-HRP to each well.

3. Incubate the plate for 30 minutes at RT with gentle agitation.

4. Wash the plate three times with 200 µl of PBS-T Buffer 1 per well.

5. Block by adding 100 µl of Blocking Buffer 7 to every well and incubate for 10 minutes at RT.

6. Tap the plate onto clean paper towel to remove the liquid.

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

8. Add 100 µl of mix to each well.

   Note: Discard any unused chemiluminescent mix after use.

9. Immediately read the plate in a plate reader capable of reading chemiluminescence.

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10. The "Blank" value should be subtracted from all readings.

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

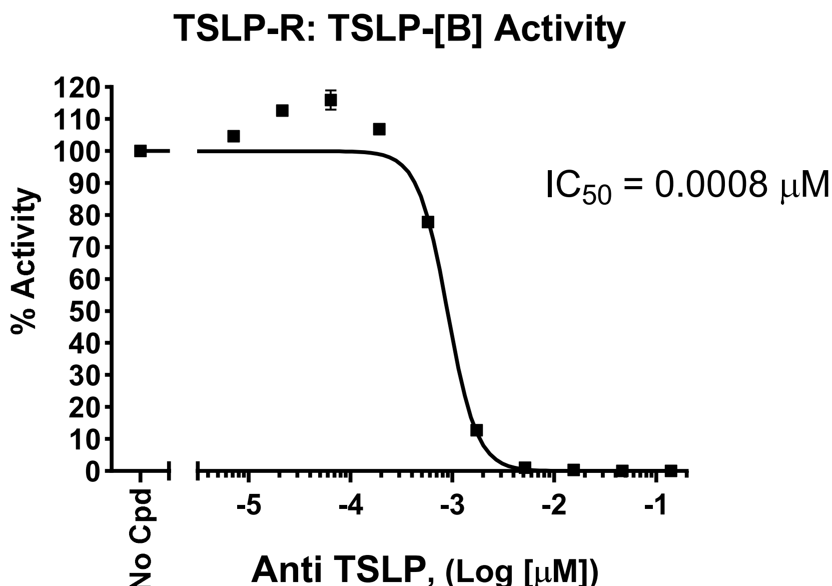

Figure 2. Inhibition of binding of TSLP to TSLPR by Anti-TSLP Neutralizing Antibody.
TSLPR:TSLP binding was evaluated in the presence of increasing concentrations of Anti-TSLP Neutralizing Antibody (#102138). Results are expressed as percent activity, in which binding activity in the absence of neutralizing antibody is set to 100%.

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

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com.

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Related Products

Products	*Catalog #*	*Size*
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 lymphopoeitin Recombinant	90250	2 µg/ 10 µg

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