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# Produktinformation

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## Zuschläge

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- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand


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# TSLPR:TSLP(R127A, R130A) [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit

#82827
96 reactions

**Description**

The TSLPR:TSLP(R127A, R130A) [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit is designed for screening and profiling small molecules that block the binding between TSLPR (thymic stromal lymphopoietin receptor) and mutant TSLP (R127A, R130A). 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.

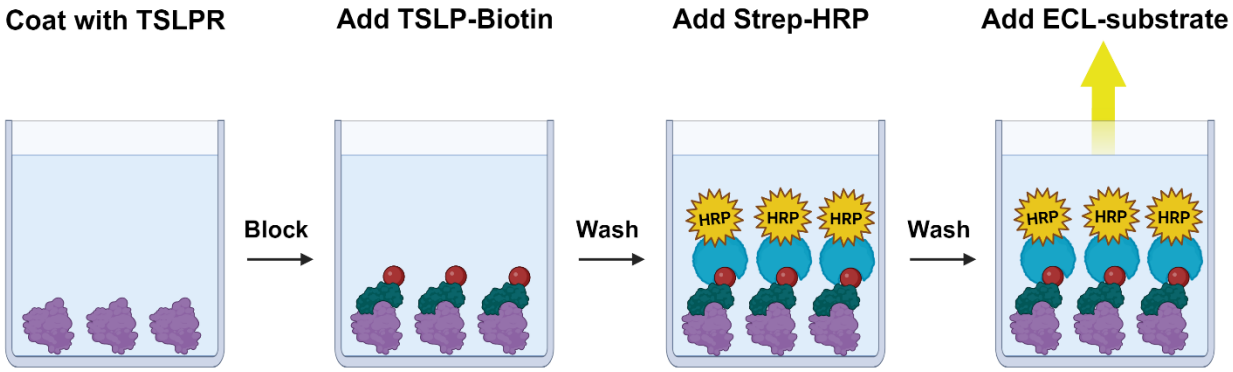


*Figure 1: Illustration of the mechanism of TSLPR: TSLP(R127A, R130A) [Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit.*

A 96-well plate is coated with TSLPR protein. After coating and blocking, TSLP(R127A, R130A) is added in an optimized assay buffer. Unbound TSLP(R127A, R130A) 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(R127A, R130A) 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 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. The mutations R127A and R130S remove a potential furin cleavage site, which may be relevant in regulating the levels of secreted TSLP.

**Application(s)**

Screen small molecule inhibitors that block TSLP(R127A, R130A) binding to TSLPR.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 102202 | TSLP (R127A, R130A), 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 Inhibitor" 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 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. For small molecule inhibitors, 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 $IC_{50}$ 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, 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 Inhibitor (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.

3.1 If the Test Inhibitor 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 Inhibitor 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 Inhibitor 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).

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

4. Add 10 µl of diluted Test Inhibitor to each well labeled as “Test Inhibitor”.

5. Add 10 µl of Diluent Solution to the “Positive Control” and “Blank” wells.

6. Thaw **TSLP(R127A, R130A)-Biotin** on ice. Briefly spin the tube containing the protein to recover its full content.

7. Dilute **TSLP(R127A, R130A)-Biotin** to 0.4 µg/ml with 1x Assay Buffer (20 µl/well).

8. Add 20 µl of diluted TSLP(R127A, R130A)-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 (18 hours) incubation at 4 °C.*

| | **Blank (non-coated wells)** | **Positive Control** | **Test Inhibitor** |
|---|---|---|---|
| 1x Assay Buffer | 20 µl | 20 µl | 20 µl |
| Diluent Solution | 10 µl | 10 µl | - |
| Test Compound | - | - | 10 µl |
| Diluted TSLP(R127A, R130A) -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**

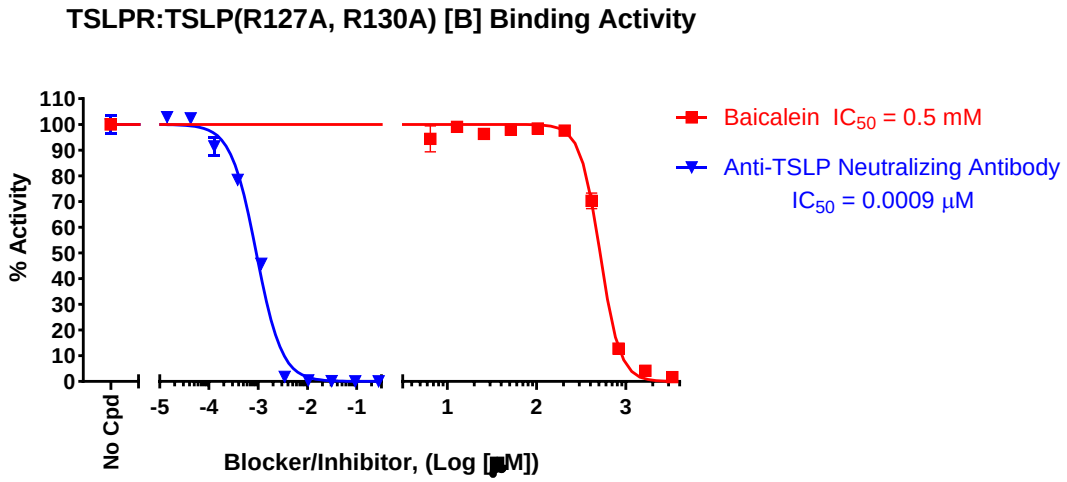


*Figure 2. Inhibition of TSLP(R127A, R130A) binding to TSLPR by Anti-TSLP Neutralizing Antibody and Baicalein.*
TSLPR:TSLP(R127A, R130A) binding was evaluated in the presence of increasing concentrations of Anti-TSLP Neutralizing Antibody (#102138) or Baicalein (Cayman #70610). Results are expressed as percent activity, in which binding activity in the absence of inhibitor/blocker is set to 100%.

*Data shown is representative. 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.

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| TSLPR: TSLP[Biotinylated] Inhibitor Screening Chemiluminescence Assay Kit | 82507 | 96 reactions |
| TSLPR: TSLP[Biotinylated] Small Molecule Inhibitor Screening Chemiluminescence Assay Kit | 82698 | 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 |

*Version 012925*