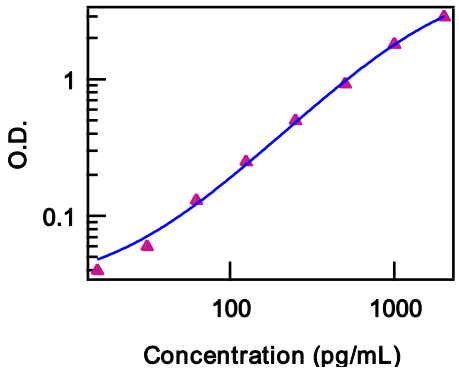
# Mouse TSLP ELISA Ready-SET-Go!®

**Catalog Number:** 88-7490
**Also known as:** Thymic Stromal-Derived Lymphopoietin
**RUO: For Research Use Only. Not for use in diagnostic procedures.**




Standard curve of Mouse TSLP ELISA Ready-SET-Go!®

## Product Information

**Contents:** Mouse TSLP ELISA Ready-SET-Go!®
REF **Catalog Number:** 88-7490
**Sensitivity:** 16 pg/mL
**Standard Curve Range:** 16-2000 pg/mL

**Temperature Limitation:** Store at 2-8°C except standard which should be stored at less than or equal to -70°C.
LOT **Batch Code:** Refer to vial
**Use By:** Refer to vial

## Description

This Mouse TSLP ELISA Ready-SET-Go! reagent set contains all of the necessary buffers and reagents to perform enzyme-linked immunosorbent assays. The set has been optimized for the accurate and precise measurement of mouse TSLP in serum, and tissue culture supernatant samples.

TSLP (Thymic Stromal-Derived Lymphopoetin) was first identified in the tissue culture supernatant of a thymic stromal cell line as a growth factor capable of inducing proliferation and differentiation of pre-B cells. It was later found to be closely related to another stromal cytokine, IL-7, with which it shares overlapping functions. Along with its proliferative effects on B cells, TSLP induces dendritic cells to support the differentiation of naïve T cells towards the Th2 lineage and may also be involved in the development of $CD4^{+}CD25^{+}$ regulatory T cells. The receptor is heterodimeric and shares one subunit, IL-7R alpha, with IL-7 and other members of the family, while the TSLPR subunit is unique to TSLP.

TSLP expression can be induced by a variety of inflammatory cytokines and TLR ligands. Expression is regulated by NF kappa B and has been detected in epithelial cells, stromal cells, and basophils. Elevated levels of TSLP are associated with asthma and atopic dermatitis, which are both Th2-mediated inflammatory conditions. It is believed that chronic overexpression of TSLP may result in increased sensitivity to allergens, resulting in susceptibility to these conditions.

## Components

**Capture Antibody**. Pre-titrated, purified antibody
**Detection Antibody**. Pre-titrated, biotin-conjugated antibody
**Standard**. Recombinant cytokine for generating standard curve and calibrating samples
**Coating Buffer**. 10X PBS ELISA Coating Buffer
**Assay Diluent**. 5X Concentrated
**Detection Enzyme**. Pre-titrated Avidin-HRP
**Substrate Solution**. Tetramethylbenzidine (TMB) Solution
**Certificate of Analysis**. Lot-specific instructions for the dilution of antibodies and standards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**96-Well Plates**. Corning Costar flat-bottom plates (included with Cat. #'s ending in -22 and -86)

**Applications Reported**
This ELISA set is for the quantitative detection of mouse TSLP in serum, plasma, and tissue culture supernatant samples.

**Applications Tested**
This assay has been validated for the detection of endogenous mouse TSLP with supernatant collected from an LPS-stimulated balb/c lung culture. Lower basal levels of TSLP were observed in unstimulated lung cultures, and no detectable levels of TSLP were observed in supernatant from stimulated and unstimulated splenocyte cultures.

This assay was evaluated for specificity on a panel of 72 recombinant cytokines at 100 ng/ml. No significant cross-reactivity was observed, including to recombinant human TSLP.

**References**
Demehri S, Morimoto M, Holtzman M, Kopan R. Skin-derived TSLP triggers progression from epidermal-barrier defects to asthma. PLoS Biol. 2009 May 19:7(5):e1000067

Taylor B, Zaph C, Troy A, Du Y, Guild K, Comeau M, Artis D. TSLP regulates intestinal immunity and inflammation in mouse models of helminth infection and colitis. J Exp Med. 2009 Mar 16;206(3):655-67

Zhou B, Headley MB, Aye T, Tocker J, Comeau MR, Ziegler SF. Reversal of thymic stromal lyphopoetin-induced airway inflammation through inhibition of Th2 responses. J Immunol. 2008 Nov 1;181(9):6557-62

**Related Products**
16-5491 Anti-Mouse TSLP Functional Grade Purified (eBio28F12 (28F12))


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# Enzyme Linked Immunosorbent Assay (ELISA)

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## Protocol: ELISA Ready-Set-Go!

The following protocol is a general guideline for the Ready-SET-Go! Sets

### Materials Provided

- Please refer to the Certificate of Analysis (C of A) for components

### Other Materials Needed

- Buffers*
  - Wash Buffer: 1x PBS, 0.05% Tween-20 (or eBioscience ELISA Wash Buffer Powder, Cat. No. 00-0400)
  - Stop Solution: 1M $H_3PO_4$ (recommended) or 2N $H_2SO_4$
- Pipettes
- Refrigerator & frost-free -20°C freezer
- 96-well plate (Corning Costar 9018 or NUNC Maxisorp®)
  ***NOTE: The use of ELISA plates which are not high affinity protein binding plates will result in suboptimal performance, e.g., low signal or inconsistent data. Do not use tissue culture plates or low protein absorption plates. Use only the Corning Costar 9018 or NUNC Maxisorp (Cat. No. 44-2404) 96-well plates***
- 96-well ELISA plate reader (microplate spectrophotometer)
- ELISA plate washer (highly recommended)

***NOTE: To ensure optimal results from this ELISA Ready-SET-Go! Set, please only use the components included in the set. Exchanging of components is not recommended as a change in signal may occur.***

### Time Requirements

- 1 overnight incubation
- 4½-hour incubations
- 1 hour washing and analyzing samples

### Experimental Procedure

1. Coat Corning Costar 9018 (or Nunc Maxisorp®) ELISA plate with 100 µL/well of capture antibody in Coating Buffer (dilute as noted on C of A, which is included with the reagent set). Seal the plate and incubate overnight at 4°C.
2. Aspirate wells and wash 3 times with >250 µL/well Wash Buffer*. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes. Blot plate on absorbent paper to remove any residual buffer.
3. Dilute 1 part 5X concentrated Assay Diluent with 4 parts DI water.* Block wells with 200 µL/well of 1X Assay Diluent. Incubate at room temperature for 1 hour.
4. Optional: Aspirate and wash at least once with Wash Buffer.
5. Using 1X Assay Diluent*, dilute standards as noted on the C of A to prepare the top concentration of the standard. Add 100 µL/well of top standard concentration to the appropriate


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wells. Perform 2-fold serial dilutions of the top standards to make the standard curve for a total of 8 points. Add 100 µL/well of your samples to the appropriate wells. Seal the plate and incubate at room temperature for 2 hours (or overnight at 4°C for maximal sensitivity).

6. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
7. Add 100 µL/well of detection antibody diluted in 1X Assay Diluent* (dilute as noted on C of A). Seal the plate and incubate at room temperature for 1 hour.
8. Aspirate/wash as in step 2. Repeat for a total of 3-5 washes**.
9. Add 100 µL/well of Avidin-HRP* diluted in 1X Assay Diluent (dilute as noted on C of A). Seal the plate and incubate at room temperature for 30 minutes.
10. Aspirate and wash as in step 2. In this wash step, soak wells in Wash Buffer* for 1 to 2 minutes prior to aspiration. Repeat for a total of 5-7 washes**.
11. Add 100 µL/well of Substrate Solution to each well. Incubate plate at room temperature for 15 minutes.
12. Add 50 µL of Stop Solution to each well.
13. Read plate at 450 nm. If wavelength subtraction is available, subtract the values of 570 nm from those of 450 nm and analyze data.

***NOTES:***

**** Be certain that no sodium azide is present in the solutions used in this assay, as this inhibits HRP enzyme activity.***

*****The number of washes in the protocol was adapted to an automatic plate washer. This can be decreased when using other methods but should be tested empirically. Allowing time for soaking (~ 1 minute) during each wash step increases the effectiveness of the washes.***


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## Quick Guide: Standard Calibration

The following table indicates the protein standard contained in the Ready-SET-Go! is calibrated against NIBSC standards.

**Table of Standard Calibration**

| Cytokine | ng of eB standard | ng of NIBSC standard | U of NIBSC standard | NIBSC Lot # |
|---|---|---|---|---|
| hIL-2 | 1 | 1.1 | 14.6 | 86/564 |
| hIL-4 | 1 | 2.2 | 22 | 88/656 |
| hIL-5 | 1 | 2.2 | 22 | 90/586 |
| hIL-6 | 1 | 1.7 | 170 | 89/548 |
| hIL-8 | 1 | 1.8 | 180 | 89/520 |
| hIL-10 | 1 | 0.8 | 4 | 93/722 |
| hIL-12 | 1 | 0.8 | 8 | 95/544 |
| hIL-17A | 1 | 0.9 | 9000 | 01/420 |
| hIFN-g | 1 | 1.1 | 22 | 87/586 |
| hTNF-a | 1 | 0.9 | 36 | 87/650 |
| mIL-2 | 1 | 3.1 | 310 | 93/566 |
| mIL-4 | 1 | 3 | 30 | 91/656 |
| mIL-6 | 1 | 8.5 | 850 | 93/730 |
| mIFN-g* | 1 | | 4.5 | Gg02-901-533 |
| mTNF-a | 1 | 1.7 | 340 | 88/532 |

* Mouse IFN-g is calibrated using NIH standard (Lot Gg02-901-533) and is measured in Units (U)

**ELISA Troubleshooting Guide**

| Problem | Possibility | Solution |
|---|---|---|
| A. High background | 1. Improper and inefficient washing | 1. Improve efficiency of washing. Fill plates completely, soak for 1 minute per wash, as directed |
| | 2. Cross contamination from other specimens or positive control | 2. Repeat ELISA being careful when washing and pipetting |
| | 3. Contaminated substrate | 3. Substrate should be colorless. Replace |
| | 4. Incorrect dilutions, e.g., conjugate concentration was too high | 4. Repeat using correct dilutions |
| B. No signal | 1. Improper, low protein binding capacity plates were used | 1. Repeat ELISA using recommended high binding capacity plates |
| | 2. Wrong substrate was used | 2. Repeat ELISA using the correct substrate |
| | 3. Enzyme inhibitor present in buffers; e.g., sodium azide in the washing buffer and Assay Diluent inhibits peroxidase activity | 3. Repeat ELISA making no enzyme inhibitor is present in any buffers. |


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| | | |
|---|---|---|
| | 4. Coated capture antibody in Assay Diluent rather than Coating Buffer | 4. Repeat ELISA using Coating Buffer contained in the set as the diluent for the capture antibody. |
| C. Very weak signal | 1. Improper and inefficient washing | 1. Make sure washing procedure is done correctly, with a soak time. |
| | 2. Incorrect dilutions of standard | 2. Follow recommendations of standard preparation exactly as written on the C of A |
| | 3. Insufficient incubation time | 3. Repeat ELISA following the protocol carefully for each step |
| | 4. Incorrect storage of reagents | 4. Store reagents at the correct temperature asn indicated on the Technical Data Sheet. Freezing certain components will severely impact results. Do not re-use the standards. |
| | 5. Wrong filter in ELISA reader was used | 5. Use the correct wavelength setting |
| | 6. Wrong plate used | 6. Use the recommended Corning Costar 9018 or NUNC Maxisorp flat bottom 96 well plates |
| D. Variation amongst replicates | 1. Improper and inefficient washing | 1. Make sure washing procedure is done correctly; see C of A. Edge effects can be avoided by moving samples and standards in from the edge of the plate. |
| | 2. Poor mixing of samples | 2. Mix samples and reagents gently and equilibrate to proper temperature |
| | 3. Plates not clean | 3. Plates should be wiped on bottom before measuring absorbance |
| | 4. Reagents have expired | 4. Order a new Ready-Set-Go ELISA. |


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