BioLegend®

The path to legendary discovery™

LEGEND MAX™

ELISA Kit with Pre-coated Plates

Human TSLP

Cat. No. 434207 1 Plate

434208 5 Plates

ELISA Kit for Accurate Quantitation of Human TSLP from Cell Culture Supernatant, Serum, Plasma and Other Biological Fluids

BioLegend, Inc.

biolegend.com

It is highly recommended that this manual be read in its entirety before using this product. Do not use this kit beyond the expiration date.

Table of Contents

Introduction:

Human TSLP (Thymic Stromal Lymphopoietin) is a hemopoietic protein that is expressed in the heart, liver and prostate. TSLP overlaps its biological activities with IL-7 and binds with the heterodimeric receptor complex consisting of the IL-7R alpha chain (IL-7Rα) and the TSLP-specific chain (TSLPR). TSLP is produced mainly by non-hematopoietic cells such as fibroblasts, epithelial cells and stromal or stromal-like cells. Human TSLP impacts myeloid cells and induces the release of T cell-attracting chemokines from monocytes and enhances the maturation of $CD11c^+$ dendritic cells. TSLP activated dendritic cells prime naïve T cells to a T_H2 phenotype, which are strong producers of cytokines IL-13, IL-5, and TNF-α. TSLP also directly stimulates the production of T_H2 cytokines by human mast cells. Finally, expression of TSLP has been linked to the pathogenesis of allergic inflammation such as atopic dermatitis and asthma via activating dendritic cells and mast cells that trigger an inflammatory T_H2 response.

BioLegend's LEGEND MAX™ Human TSLP ELISA Kit is a Sandwich Enzyme-Linked Immunosorbent Assay (ELISA) with a 96-well strip plate that is pre-coated with a capture antibody. This is specifically designed for the accurate quantitation of human from cell culture supernatant, serum, plasma and other biological fluids. All kits are analytically validated with ready-to-use reagents.

Materials Provided:

Description	Quantity (1 plate)	Quantity (5 plates)	Volume (per bottle)	Part #
Anti-human TSLP pre-coated 96-well Strip Microplate	1 plate	5 plates		78216
Human TSLP Detection Antibody	1 bottle	5 bottles	12 mL	78305
Human TSLP Standard	1 vial	5 vials	lyophilized	78304
Avidin-HRP A	1 bottle	5 bottles	12 mL	79131
Assay Buffer A	1 bottle	5 bottles	25 mL	78232
Wash Buffer (20X)	1 bottle	5 bottles	50 mL	78233
Substrate Solution F	1 bottle	5 bottles	12 mL	79132
Stop Solution	1 bottle	5 bottles	12 mL	79133
Plate Sealers	4 sheets	20 sheets		78101

Tel: 858-768-5800

Materials to be Provided by the End-User:

- Microplate reader able to measure absorbance at 450 nm
- Adjustable pipettes to measure volumes ranging from 1 µL to 1,000 µL
- Deionized water
- Wash bottle or automated microplate washer
- Log-Log graph paper or software for data analysis
- Tubes to prepare standard dilutions
- Timer
- Plate Shaker
- Polypropylene Vials

Storage Information:

Store unopened kit components at 4°C. Do not use this kit beyond its expiration date.

<table>
<tr><th colspan="2">Opened or Reconstituted Components</th></tr>
<tr><td>Microplate wells</td><td>If not all microplate strips are used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal. Store at 4°C for up to one month.</td></tr>
<tr><td>Standard</td><td>The remaining reconstituted standard stock solution can be aliquoted into polypropylene vials and stored at -70°C for up to one month. Avoid repeated freeze-thaw cycles.</td></tr>
<tr><td>Detection Antibody</td><td rowspan="6">Store opened reagents at 4°C and use within one month.</td></tr>
<tr><td>Avidin-HRP A</td></tr>
<tr><td>Assay Buffer A</td></tr>
<tr><td>Wash Buffer (20X)</td></tr>
<tr><td>Substrate Solution F</td></tr>
<tr><td>Stop Solution</td></tr>
</table>

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online at BioLegend's website for details (www.biolegend.com/msds).
2. Substrate Solution F is harmful if inhaled or ingested. Avoid skin, eye and clothing contact.
3. To reduce the likelihood of blood-borne transmission of infectious agents,

handle all serum, plasma and other biological fluids in accordance with NCCLS regulations.

4. Stop Solution contains strong acid. *Wear eye, hand, and face protection.*
5. Before disposing of the plate, rinse with an excess amount of tap water.

Specimen Collection and Handling:

Specimens should be clear and non-hemolyzed. If possible, unknown samples should be run at a number of dilutions to determine the optimal dilution factor that will ensure accurate quantitation.

Cell Culture Supernatant: If necessary, centrifuge all samples to remove debris prior to analysis. It is recommended that samples be stored at < -70°C. Avoid repeated freeze-thaw cycles.

Serum: Use a serum separator tube and allow clotting for at least 30 minutes, then centrifuge for 10 minutes at 1,000 x *g*. Remove serum layer and assay immediately or store serum samples at < -70°C. Avoid repeated freeze-thaw cycles.

Plasma: Collect blood samples in citrate, heparin or EDTA containing tubes. Centrifuge for 10 minutes at 1,000 x *g* within 30 minutes of collection. Assay immediately or store plasma samples at < -70°C. Avoid repeated freeze-thaw cycles.

Reagent and Sample Preparation:

Note: All reagents should be diluted immediately prior to use.

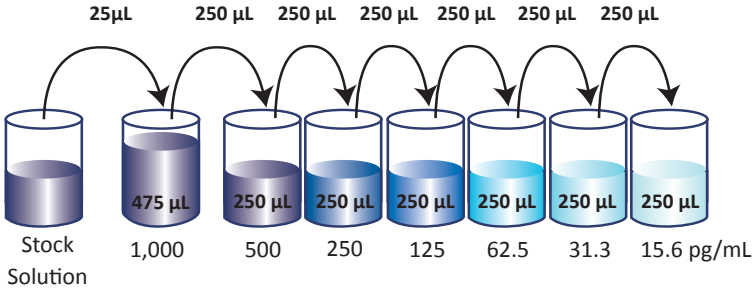
1. Dilute the 20X Wash Buffer to 1X with deionized water. For example, make 1 liter of 1X Wash Buffer by adding 50 mL of 20X Wash Buffer to 950 mL of deionized water. If crystals have formed in the 20X Wash Buffer, bring to room temperature and vortex until dissolved.
2. Reconstitute the lyophilized Human TSLP Standard by adding the volume of Assay Buffer A indicated on the vial label to make the 20 ng/mL standard stock solution. Allow the reconstituted standard to sit at room temperature for 15 minutes, then briefly vortex to mix completely.
3. In general, serum, plasma and cell culture supernatant samples are analyzed without dilutions. However, if dilutions are required, use Assay Buffer A as the sample diluent.

Tel: 858-768-5800

Assay Procedure:

Note: Do not mix reagents from different kits or lots. Reagents and/or antibodies from different manufacturers should not be used with this kit.

1. Bring all reagents to room temperature prior to use. It is strongly recommended that all standards and samples be run in duplicate or triplicate. A standard curve is required for each assay.
2. If not all microplate strips will be used, remove the excess strips by pressing up from underneath each strip. Place excess strips back in the foil pouch with the included desiccant pack and reseal.
3. Prepare 500 µL of the 1,000 pg/mL top standard by diluting 25 µL of the standard stock solution in 475 µL of Assay Buffer A. Perform six two-fold serial dilutions of the 1,000 pg/mL top standard in separate tubes using Assay Buffer A as the diluent. Thus, the human TSLP standard concentrations in the tubes are 1,000 pg/mL, 500 pg/mL, 250 pg/mL, 125 pg/mL, 62.5 pg/mL, 31.3 pg/mL and 15.6 pg/mL respectively. Assay Buffer A serves as the zero standard (0 pg/mL).



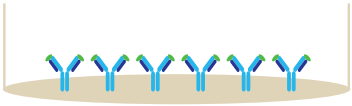
4. Wash the plate 4 times with at least 300 µL of 1X Wash Buffer per well and blot any residual buffer by firmly tapping the plate upside down on absorbent paper. All subsequent washes should be performed similarly.
5. Add 50 µL of Assay Buffer A to each well that will contain either standard dilutions or samples.
6. Add 50 µL of standard dilutions or samples to the appropriate wells.
7. Seal the plate with a Plate Sealer included in the kit and incubate the plate at room temperature for 2 hours while shaking at 200 rpm.
8. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.

9. Add 100 µL of Human TSLP Detection Antibody solution to each well, seal the plate and incubate at room temperature for 1 hour while shaking.
10. Discard the contents of the plate into a sink, then wash the plate 4 times with 1X Wash Buffer as in step 4.
11. Add 100 µL of Avidin-HRP A solution to each well, seal the plate and incubate at room temperature for 30 minutes while shaking.
12. Discard the contents of the plate into a sink, then wash the plate 5 times with 1X Wash Buffer as in step 4. For this final wash, soak wells in 1X Wash Buffer for 30 seconds to 1 minute for each wash. This will help minimize background.
13. Add 100 µL of Substrate Solution F to each well and incubate for 10 minutes in the dark. Wells containing human TSLP should turn blue in color with an intensity proportional to its concentration. It is not necessary to seal the plate during this step.
14. Stop the reaction by adding 100 µL of Stop Solution to each well. The solution should change from blue to yellow.
15. Read absorbance at 450 nm within 30 minutes. If the reader is capable of reading at 570 nm, the absorbance at 570 nm can be subtracted from the absorbance at 450 nm.

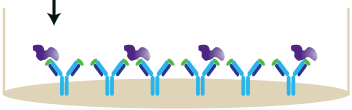
Tel: 858-768-5800

Assay Procedure Summary

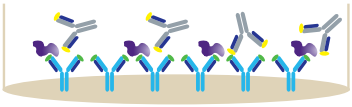
1. Wash 4 times
Add 50 µL Assay Buffer A



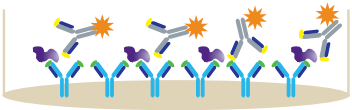
2. Add 50 µL diluted standards or samples
Incubate 2 hr, RT, shaking



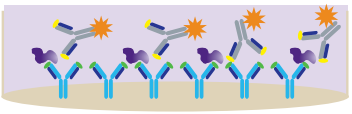
3. Wash 4 times
Add 100 µL Detection Antibody solution
Incubate 1 hr, RT, shaking



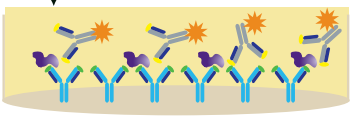
4. Wash 4 times
Add 100 µL Avidin-HRP A solution
Incubate 30 min, RT, shaking



5. Wash 5 times
Add 100 µL Substrate Solution F
Incubate 10 min, RT, in the dark



6. Add 100 µL Stop Solution



7. Read absorbance at 450 nm and 570 nm

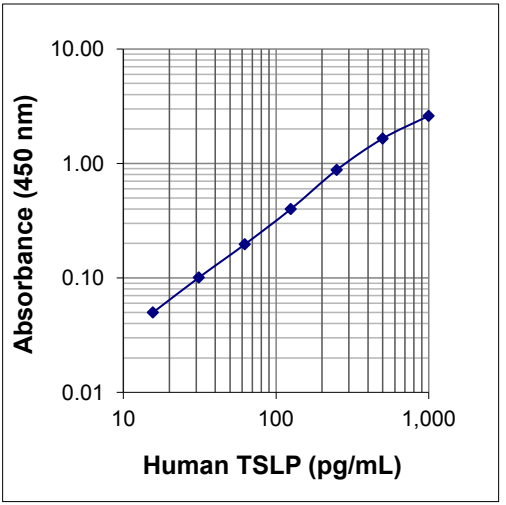
Calculation of Results:

The data can be best calculated with computer-based curve-fitting software using a 5- or 4-parameter logistics curve-fitting algorithm. If an appropriate software is not available, use log-log graph paper to determine sample concentrations. Determine the mean absorbance for each set of duplicate or triplicate standards, controls, and samples. Plot the standard curve on log-log graph paper with cytokine concentration on the X-axis and absorbance on the Y-axis. Draw a best fit line through the standard points. To determine the unknown cytokine concentrations, find the mean absorbance value of the unknown concentration on the Y-axis and draw a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the cytokine concentration.

If samples were diluted, multiply the concentration by the appropriate dilution factor. If a test sample's absorbance value falls outside the linear portion of the standard curve, the test sample needs to be re-analyzed at a higher (or lower) dilution as appropriate.

Typical Data:

This standard curve was generated at BioLegend for demonstration purposes only. A standard curve must be run with each assay.



Performance Characteristics:

Specificity: No cross-reactivity was observed when this kit was used to analyze mouse TSLP and the following recombinant cytokines/chemokines at up to 50 ng/ml.

Human	IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-11, IL-12/IL-23 (p40), IL-12(p70), IL-13, IL-15, IL-17A/F, IL-17E, IL-22, IL-23, IL-27, FGF-basic, G-CSF, IFN-γ, MCP-1/CLL2, RANTES/CCL5, SDF-1α, TGF-β1, TNF-α, TNF-β, TWEAK, VEGF-165

Sensitivity: The minimum detectable concentration of TSLP is 1 pg/mL.

Recovery: Human TSLP (500, 250, 125, 62.5, 31.3, 15.6 and 7.8 pg/mL) was spiked into human serum samples, and then analyzed with the LEGEND MAX™ Human TSLP kit. On average, 92.6% of the cytokine was recovered from serum samples.

Linearity: Three serum samples with high concentrations of TSLP were diluted 1:1, 1:3, 1:7, 1:15, and 1:31 with Assay Buffer A to produce samples with values within the dynamic range and then assayed. On average, 99% of the expected cytokine was detected from diluted serum samples.

Intra-Assay Precision: Sixteen replicates of each of two samples containing different TSLP concentrations were tested in one assay.

Concentration	Sample 1	Sample 2
Number of Replicates	16	16
Mean Concentration (pg/mL)	59.6	16.9
Standard Deviation	2.08	0.78
%CV	3.5	4.6

Inter-Assay Precision: Two samples containing different concentrations of TSLP were tested in three independent assays.

Concentration	Sample 1	Sample 2
Number of Assays	3	3
Mean Concentration (pg/mL)	58.8	15.6
Standard Deviation	0.64	1.74
%CV	1.9	11.2

Biological Samples: Human primary bronchial cells were pre-stimulated with DMSO and subsequently stimulated with double-stranded RNA (dsRNA) or dsRNA and IL-4 for 48 hours. The dsRNA functions as a toll-like receptor 3 stimulant. Then the cell culture supernatant was collected and the TSLP concentration was measured with the assay. Double-stimulated cell media had a mean concentration of 53 pg/mL, while single-stimulated cell media had 22 pg/mL, and the unstimulated cell media had no detectable TSLP.

Troubleshooting Guide:

Problem	Probable Cause	Solution
High Background	Background wells were contaminated	Avoid cross-well contamination by using the provided plate sealers. Use multichannel pipettes and change tips between pipetting samples and reagents.
	Insufficient washes	Increase number of washes. Increase soaking time between washes prior to addition of substrate solution.
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells. Use a clean container prior to pipetting substrate solution into wells.
No or poor signal	Detection Antibody, Avidin-HRP or Substrate solution were NOT added	Rerun the assay and follow the protocol.
	Wrong reagent or reagents were added in wrong sequential order	
	Insufficient plate agitation	The plate should be agitated during all incubation steps using a plate shaker at a speed where solutions in wells are within constant motion without splashing.
	The wash buffer contains Sodium Azide (NaN3)	Avoid Sodium Azide contamination in the wash buffer as it inhibits HRP activity.
	Incubations were done at an inappropriate temperature, timing or without agitation	Rerun the assay and follow the protocol.
Low or poor standard curve signal	The standard was incorrectly reconstituted or diluted	Adjust the calculations and follow the protocol.
	Standard was inappropriately stored	Store the reconstituted standard stock solution in polypropylene vials at -70°C. Avoid repeated freeze-thaw cycles.
	Reagents added to wells with incorrect concentrations	Check for pipetting errors and the correct reagent volume.

Tel: 858-768-5800

Problem	Probable Cause	Solution
Signal is high, standard curves have saturated signal	Standard reconstituted with less volume than required	Reconstitute new lyophilized standard with the correct volume of solution recommended in the protocol.
	Standards/samples, detection antibody, Avidin-HRP or substrate solution were incubated for too long	Rerun the assay and follow the protocol.
Sample readings are out of range	Samples contain no or below detectable levels of the analyte	If samples are below detectable levels, it may be possible to use a larger sample volume. Contact technical support for appropriate protocol modifications.
	Samples contain analyte concentrations greater than highest standard point	Samples may require dilution and analysis.
High variation in samples and/or standards	Multichannel pipette errors	Confirm that pipette calibrations are accurate.
	Plate washing was not adequate or uniform	Ensure pipette tips are tightly secured. Ensure uniformity in all wash steps.
	Non-homogenous samples	Thoroughly mix samples before assaying.
	Samples may have high particulate matter	Remove particulate matter by centrifugation.
	Cross-well contamination	Do not reuse plate sealers. Always change tips for reagent additions. Ensure that pipette tips do not touch the reagents on the plate.

ELISA Plate Template

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Tel: 858-768-5800

Notes

LEGEND MAX™ Kits are manufactured by **BioLegend Inc.**
9727 Pacific Heights Blvd.
San Diego, CA 92121
Tel: 1.858.768.5800
Tel: US & Canada Toll-Free: 1.877.Bio-Legend (1.877.246.5343)
Fax: 1.877.455.9587
Email: info@biolegend.com
biolegend.com

For a complete list of world-wide BioLegend offices and distributors, please visit our website at: biolegend.com

78984-REV002-05022013