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Human TSLP Immunoassay

Catalog Number: SEKH-0334

For the quantitative determination of human TSLP concentrations in cellculture supernates, serum, and plasma.

For research use only. Not for use in diagnostic procedures.

MANUFACTURED AND DISTRIBUTED BY:

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TABLE OF CONTENTS

REPEATABILITY: The coefficient of variation of both intra-assay and inter-assay were less than 10%.

RECOVERY: The recovery of TSLP spiked to three different levels in four samples throughout the range of the assay in various matrices was evaluated.

Recovery of TSLP two matrices

Sample Type	Average % of Expected Range (%)	Range (%)
Citrate plasma	96	85—107
Cell culture supernatants	98	90—106

LINEARITY: To assess the linearity of the assay, three samples were spiked with high concentrations of TSLP in various matrices and diluted with the appropriate Sample Diluent to produce samples with values within the dynamic range of the assay. (The plasma samples were initially diluted 1:1)

Dilution ratio	Recovery(%)	Citrate plasma	Cell culture supernatants
1:2	Average% of Expected	96	101
	Range (%)	89—104	90—112
1:4	Average% of Expected	97	103
	Range (%)	90—104	94—112
1:8	Average% of Expected	99	102
	Range (%)	88—110	93—111
1:16	Average% of Expected	98	104
	Range (%)	89—107	96—112

Performance Characteristics

SENSITIVITY: The minimum detectable dose was 15.63 pg/mL.
SPECIFICITY: This assay recognizes both natural and recombinant human TSLP. The factors listed below were prepared at 100ng/ml in Standard /sample Diluent and assayed for cross-reactivity and no significant cross-reactivity or interference was observed.

Factors assayed for cross-reactivity

Recombinant human	Recombinant mouse	Recombinant porcine
ANG	Cardiotrophin-1	
Ang-2	CTLA-4	
Amphiregulin	Fas	
BDNF	Fas Ligand	
CD4	IFN-γ	
CD40	IL-1α	
Ligand CNTF	IL-1β	
Cardiotrophin-1	IL-1ra	
CTLA-4	IL-2	
	IL-3	

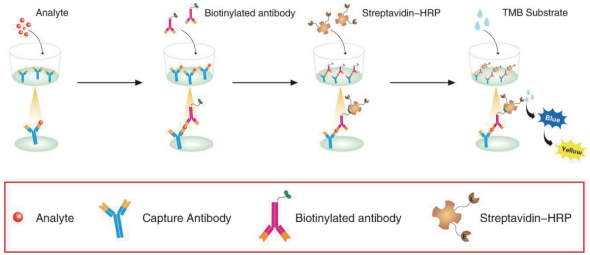
BACKGROUND

Thymic Stromal Lymphopoietin (TSLP) is a 23 kDa member of the IL-7 family of α-helical cytokines . It is a monomeric glycoprotein that is synthesized as a 159 amino acid (aa) precursor. Human TSLP contains a 28 aa signal sequence plus a 131 aa mature segment. The mature molecule contains four α-helices, six cysteines, and two potential N-linked glycosylation sites. One isoform may exist that is 63 aa in length. It appears to be the product of an alternate start site at Met 96. If translated, it would show an absence of the signal sequence plus the first two α-helices of the full-length form. Human TSLP shows significant divergence from mouse TSLP. The mouse TSLP precursor is only 140 aa in length with a 19 aa signal sequence and a 121 aa mature region. In the mature region, human TSLP shares 37% aa sequence identity with mouse TSLP. The mature human TSLP receptor (TSLP R; also known as CRLF2) is a type I transmembrane glycoprotein that is 348 aa in length. It contains a 208 aa extracellular region, a 21 aa transmembrane segment, and a 119 aa cytoplasmic domain. Although it belongs to the hematopoietin (type I cytokine) receptor superfamily, its extracellular region lacks one of the eight canonical cysteines, suggesting an unusual folding pattern. It binds TSLP with low affinity and does not bind IL-7 at all. When complexed to IL-7 Rα, TSLP R activates STAT5 but not Jaks. It is proposed that Tec protein kinases substitute for the Jak kinase system.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for TSLP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells; any TSLP present is captured by the coated antibody after incubation. After washing away any unbound substances, a biotin-conjugate antibody specific for TSLP is added to detect the captured TSLP protein in the sample. Following a wash to remove any unbound combination, horseradish peroxidase (HRP)-conjugated Streptavidin is added to the wells. After extensive washing, a tetramethyl-benzidine (TMB) reagent is added to the wells for signal development. Solution containing sulfuric acid is used to stop color development. The color intensity, proportional to the quantity of bound protein, is then measurable at 450nm.

Schematic diagram:



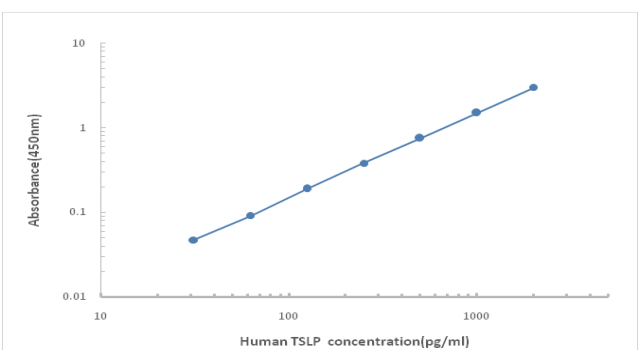
TECHNICAL HINTS AND LIMITATIONS

1.This Solarbio ELISA should not be used beyond the expiration data on the kit label.
2.To avoid cross-contamination, use a fresh reagent reservoir and pipette tips for each step.
3.To ensure accurate results, some details, such as technique, plasticware and water sources should be emphasized.
4.A thorough and consistent wash technique is essential for proper assay performance.
5.A standard curve should be generated for each set of samples assayed.
6.It is recommended that all standards and samples be assayed in duplicate.
7.Avoid microbial contamination of reagents and buffers. Buffers containing protein should be made under aseptic conditions and be prepared fresh daily.
8.In order to ensure the accuracy of the results, the standard curve should be made every time.

read from the standard curve must be multiplied by the dilution factor.
5. This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

Typical data using the TSLP ELISA

Standard(pg/ml)	O.D.1	O.D.2	Average	Corrected
0	0.073	0.069	0.071	---
31.25	0.168	0.164	0.166	0.095
62.5	0.277	0.272	0.275	0.204
125	0.462	0.459	0.461	0.390
250	0.814	0.810	0.812	0.741
500	1.450	1.448	1.449	1.378
1000	2.385	2.381	2.383	2.312
2000	3.141	3.138	3.140	3.069



Representative standard curve for TSLP ELISA.

ASSAY PROCEDURE

Prepare all reagents and standards as directed.Wash the plate 3 times before assay.

⇩

Add 100μl standard or samples to each well, incubate 90 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Biotin-Conjugate anti-human TSLP antibody to each well, incubate 60 minutes,37℃.

⇩ Aspirate and wash 4 times

Add 100μl working solution of Streptavidin-HRP to each well, incubate 30 minutes,37℃.

⇩ Aspirate and wash 5 times

Add 100μl Substrate solution to each well, incubate 15 minutes, 37℃.Protect from light.

⇩

Add 50μl Stop solution to each well. Read at 450nm within 5 minutes.

CALCULATION OF RESULTS

1. The standard curve is used to determine the amount of specimens.
2. First, average the duplicate readings for each standard, control, and sample.All O.D. values are subtracted by the mean value of blank control before result interpretation.
3. Construct a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph.
4. The data may be linearized by plotting the log of the TSLP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data. If samples have been diluted, the concentration

PRECAUTIONS

The Stop Solution suggested for use with this kit is an acid solution. Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling.

KIT COMPONENTS& STORAGE CONDITIONS

PART	SIZE	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Microwell Plate-antibody coated 96-well Microplate (8 wells ×12 strips)	1 plate	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2 – 8℃**
Standard - lyophilized,2000 pg/ml upon reconstitution	2 vials	Store at 2-8°C **for six months
ConcentratedBiotin-Conjugated antibody(100X) - 120 ul/vial	1 vial	Store at 2-8°C **for six months
ConcentratedStreptavidin-HRP solution(40X) - 300 ul/vial	1 vial	Store at 2-8°C **for six months
Standard /sample Diluent - 16ml/vial	1 bottle	Store at 2-8°C **for six months
Biotin-Conjugate antibody Diluent- 16ml/vial	1 bottle	Store at 2-8°C **for six months
Streptavidin-HRP Diluent - 16ml/vial	1 bottle	Store at 2-8°C **for six months
Wash Buffer Concentrate (20x) - 30 ml/vial	1 bottle	Store at 2-8°C **for six months
Substrate Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Stop Solution - 12 ml/vial	1 bottle	Store at 2-8°C **for six months
Plate Cover Seals	4 pieces	

**Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED BUT NOT SUPPLIED

1.Microplate reader capable of measuring absorbance at 450 nm.
2.Pipettes and pipette tips.
3.Deionized or distilled water.
4.Squirt bottle, manifold dispenser, or automated microplate washer.
5.500 mL graduated cylinder.

SPECIMEN COLLECTION & STORAGE

Cell Culture Supernates- Centrifuge cell culture media at 1000×gto remove debris. Assay immediately oraliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 2hours at roomtemperature or overnight at 2-8℃.Centrifugeapproximately for 15 minutes at 1000×g. Assayimmediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

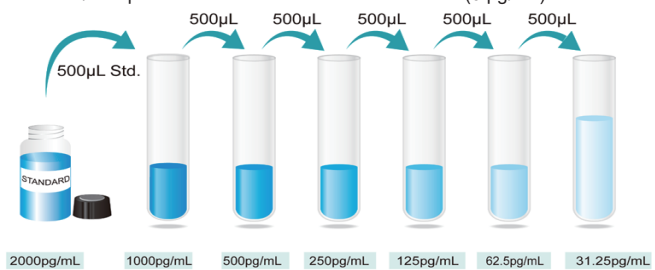
Plasma - Collect plasma using EDTA, heparin, or citrate as an anticoagulant. Centrifuge for15 minutes at 1000×g within 30 minutes of collection. Assay immediately or aliquot and storesamples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: It is recommended to conduct a pre-test before the formal experiment to determine the dilution ratio.

REAGENTS PREPARATION

1. **Temperature returning** - Bring all kit components and specimen to room temperature (20-25℃) before use.
2. **Wash Buffer** - Dilute 30mL of Wash Buffer Concentratewith 570mL of deionized or distilled water to prepare 600mL of Wash Buffer. If crystals have formed in the concentrate Wash Buffer, warm to room temperature and mixgently until the crystals have completely dissolved.
3. **Standard\Sample**- Reconstitute the Standard with 1.0mL of Standard/Sample Diluent. This reconstitution produces a stock solution of 2000pg/mL. Allow the standard to sit for a minimum of 15 minutes with

gentle agitation prior to making dilutions. Pipette 500 μL of Standard/Sample Diluent into 1000pg/ml tube and the remaining tubes. Use the stock solution of 2000pg/mL to produce a 2-folddilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2000pg/mL standard serves as the high standard. The Standard/Sample Diluent serves as the zero standard (0 pg/mL).



Preparation of TSLP standard dilutions

***If you do not run out of re-melting standard, store it at -20℃. Diluted standard shall not be reused.**

4. **Working solution of Biotin-Conjugate anti-human TSLP antibody:** Make a 1:100 dilution of the concentrated Biotin-Conjugate solution with the Biotin-Conjugate antibody Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**
5. **Working solution of Streptavidin-HRP:** Make a 1:40 dilution of the concentrated Streptavidin-HRP solution with the Streptavidin-HRP Diluent in a clean plastic tube.
   ***The working solution should be used within one day after dilution.**