Quantikine® ELISA

Mouse TSLP Immunoassay

Catalog Number MTLP00

For the quantitative determination of mouse Thymic Stromal Lymphopoietin (TSLP) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Read this package insert in its entirety before using this product.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

TABLE OF CONTENTS

MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

Mouse TSLP (thymic stromal lymphopoietin) is a 24 kDa, monomeric glycoprotein that was originally identified as a product of a thymic stromal cell line (1). It is synthesized as a 140 amino acid (aa) precursor that contains a 19 aa signal sequence plus a 121 aa mature segment. The molecule exists as a four α-helix type I cytokine that contains seven cysteines and two potential N-linked glycosylation sites. Based on its structure, TSLP is classified as a member of the IL-2 family. Human TSLP shows significant sequence and functional divergence from mouse TSLP. The human TSLP precursor is 159 aa in length with a 28 aa signal sequence and a 131 aa mature region (2). In the mature region, human TSLP shares only 38% aa sequence identity with mouse TSLP (2). Mouse TSLP is not active on human cells (3).

A high affinity heteromeric receptor complex for TSLP has been reported. It is composed of a 50 kDa low-affinity TSLP receptor (TSLP R) and the 70 kDa IL-7 receptor-alpha (IL-7 Rα) (3-5). The mature TSLP R is a type I transmembrane glycoprotein that is 349 aa in length. It contains a 222 aa extracellular region, a 21 aa transmembrane segment, and a 106 aa cytoplasmic domain (3). Although it belongs to the hematopoietin receptor superfamily, its extracellular region lacks both the typical WSxWS motif and one of the eight canonical cysteines (3, 5). It binds TSLP with low affinity, but does not bind IL-7 at all (3). When complexed with IL-7 Rα, it induces tyrosine phosphorylation of STAT5 without activation of the Janus family kinases (Jaks) (6). Two alternate TSLP R splice forms exist, one showing an 11 aa deletion in the membrane proximal area, and the other a soluble 35 kDa isoform (7, 8). Their functions are unknown.

TSLP has species-specific functions. In the mouse, TSLP is reported to impact B cell maturation at different stages *in vitro*. In general, pro-B cells give rise to large (cycling) pre-B cells that transform into small pre-B cells, which generate immature $B220^+$ IgM^+ B cells. In fetal liver, TSLP promotes the proliferation of fetal pro-B cells (9, 10). In adult bone marrow, precursor B cells known to respond to TSLP are limited to large pre-B cells that express the pre-B cell receptor complex (9). Although adult pro-B cells express a TSLP R:IL-7 Rα complex, they fail to respond to TSLP. The molecular basis for the differential responses between fetal liver and bone marrow pro-B cells to TSLP remains to be determined (10). TSLP has also been shown to expand the $CD4^+$ subset of lymphocytes and thymocytes and thus regulate $CD4^+$ homeostasis (11). When over-expressed, TSLP causes imbalances in lymphopoiesis and myelopoiesis (12). Since no effects on B cell lymphopoiesis is evident in TSLP R knockout mice (13), the exact *in vivo* functions of TSLP remains to be determined. The principal function attributed to human TSLP is one of dendritic cell regulation, an effect not seen in mice (14-16).

The Quantikine® Mouse TSLP Immunoassay is a 4.5 hour solid-phase ELISA designed to measure mouse TSLP in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant mouse TSLP and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural mouse TSLP showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural mouse TSLP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse TSLP has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any TSLP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for mouse TSLP is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of TSLP bound in the initial step. The sample values are then read off the standard curve.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse TSLP Microplate	892852	96 well polystyrene microplate (12 strips of 8 wells) coated with monoclonal antibody specific for mouse TSLP.	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 °C.*
Mouse TSLP Conjugate	892853	12 mL of a monoclonal antibody specific for mouse TSLP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Mouse TSLP Standard	892854	Recombinant mouse TSLP in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Mouse TSLP Control	892855	Recombinant mouse TSLP in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1-21	895215	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5T	895175	21 mL of a buffered protein solution with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	23 mL of diluted hydrochloric acid.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Test tubes for dilution of standards.

PRECAUTIONS

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Plasma - Collect plasma using EDTA or heparin as an anticoagulant. Centrifuge for 20 minutes at 2000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Citrate plasma has not been validated for use in this assay.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

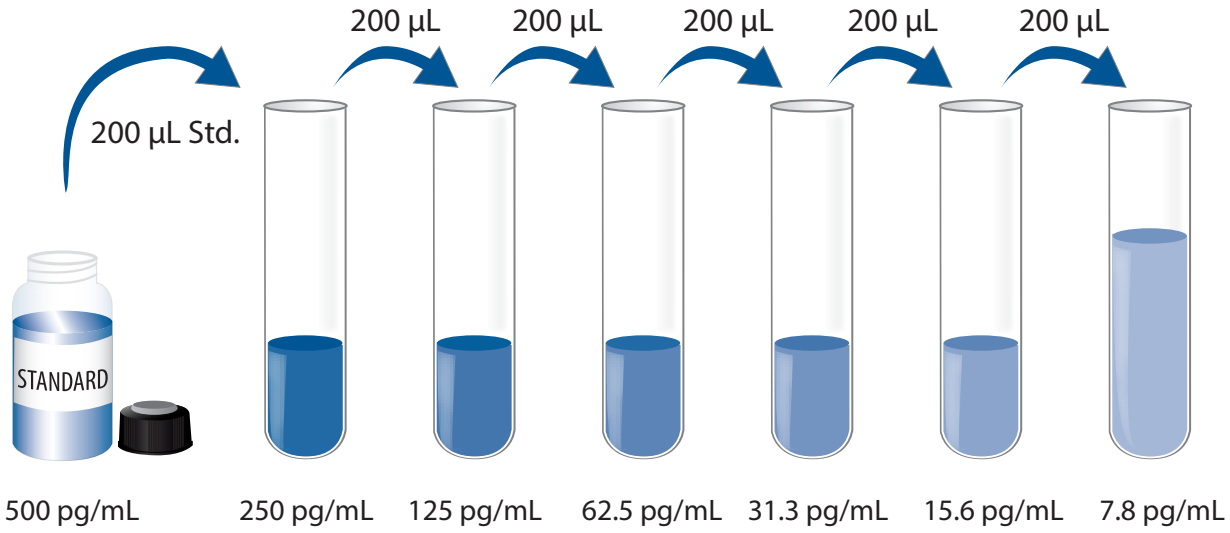
Mouse TSLP Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 100 µL of the resultant mixture is required per well.

Mouse TSLP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse TSLP Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Mouse TSLP Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5T serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standard dilutions, control, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-21 to each well.
4. Add 50 µL of standard, control, or sample per well. Mix by gently tapping the plate frame for 1 minute. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on the benchtop.
5. Aspirate each well and wash, repeating the process four times for a total of five washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Mouse TSLP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 100 µL of Stop Solution to each well. Gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

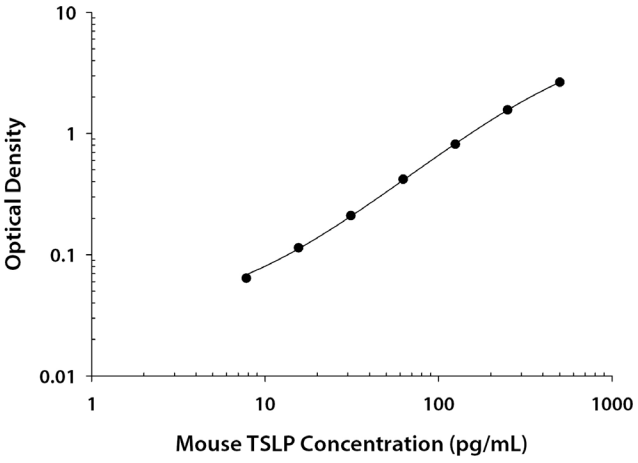
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create 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. The data may be linearized by plotting the log of the mouse 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 read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.044 0.044	0.044	—
7.8	0.104 0.112	0.108	0.064
15.6	0.155 0.160	0.158	0.114
31.3	0.251 0.257	0.254	0.210
62.5	0.453 0.472	0.463	0.419
125	0.839 0.876	0.858	0.814
250	1.610 1.611	1.611	1.567
500	2.689 2.690	2.690	2.646

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in thirty-one separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	31	31	31
Mean (pg/mL)	45	70	167	50	84	189
Standard deviation	2.5	3.2	8.5	3.8	8.2	12.9
CV (%)	5.6	4.6	5.1	7.6	9.8	6.8

RECOVERY

The recovery of mouse TSLP spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	103	93-114%
Serum (n=5)	92	87-101%
EDTA plasma (n=4)	94	80-108%
Heparin plasma (n=5)	96	89-119%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of mouse TSLP in each matrix were diluted with calibrator diluent and then assayed.

		Cell culture supernates (n=6)	Serum (n=5)	EDTA plasma (n=4)	Heparin plasma (n=5)
1:2	Average % of Expected	101	107	106	100
	Range (%)	97-103	105-109	105-109	85-106
1:4	Average % of Expected	101	109	108	105
	Range (%)	94-105	106-113	106-112	98-110
1:8	Average % of Expected	102	113	111	110
	Range (%)	92-107	109-120	109-115	102-115
1:16	Average % of Expected	103	116	115	113
	Range (%)	90-108	112-120	113-120	107-120

SENSITIVITY

Twenty-three assays were evaluated and the minimum detectable dose (MDD) of mouse TSLP ranged from 0.71-6.3 pg/mL. The mean MDD was 2.63 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant mouse TSLP produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Ten individual mouse serum, EDTA plasma, and heparin plasma samples were evaluated for detectable levels of mouse TSLP in this assay. Most samples read below the low standard, 7.8 pg/mL. Three serum samples read 8.5 pg/mL, 8.2 pg/mL, and 16.2 pg/mL. Two EDTA plasma samples read 9.3 pg/mL and 12.2 pg/mL. One heparin plasma sample read 10.6 pg/mL.

Cell Culture Supernates:
Lungs from one mouse were chopped into 1-2 mm pieces and seeded into approximately 30 mL of media containing RPMI, 10% fetal bovine serum, 50 µM β-mercaptoethanol, 10 µg/mL Concanavalin A and L-glutamine. The cell culture supernate was removed after three days, tested for mouse TSLP, and measured 114 pg/mL.

A heart from one mouse was chopped into 1-2 mm pieces and seeded into approximately 30 mL of media containing RPMI, 10% fetal bovine serum, 50 µM β-mercaptoethanol and L-glutamine. The cell culture supernate was removed after three days, tested for mouse TSLP, and measured 150 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse TSLP.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range mouse TSLP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IL-2
IL-4
IL-7
IL-7 Rα
IL-9
IL-13
IL-15
IL-21
TSLP R

Recombinant human:

IL-7
TSLP
TSLP R

REFERENCES

1. Sims, J.E. *et al.* (2000) J. Exp. Med. **192**:671.
2. Reche, P.A. *et al.* (2001) J. Immunol. **167**:336.
3. Park, L.S. *et al.* (2000) J. Exp. Med. **192**:659.
4. Leonard, W.J. (2002) Nat. Immunol. **3**:605.
5. Pandey, A. *et al.* (2000) Nat. Immunol. **1**:59.
6. Levin, S.D. *et al.* (1999) J. Immunol. **162**:677.
7. Blagoev, B. *et al.* (2001) Gene **284**:161.
8. Hiroyama, T. *et al.* (2000) Biochem. Biophys. Res. Commun. **272**:224.
9. Vosshenrich, C.A.J. *et al.* (2004) Proc. Natl. Acad. Sci. USA **101**:11070.
10. Vosshenrich, C.A.J. *et al.* (2003) Nat. Immunol. **4**:773.
11. Al-Shami, A. *et al.* (2004) J. Exp. Med. **200**:159.
12. Osborn, M.J. *et al.* (2004) Blood **103**:843.
13. Carpino, N. *et al.* (2004) Mol. Cell. Biol. **24**:2584.
14. Gilliet, M. *et al.* (2003) J. Exp. Med. **197**:1059.
15. Soumelis, V. *et al.* (2002) Nat. Immunol. **3**:673.
16. Watanabe, N. *et al.* (2004) Nat. Immunol. **5**:426.