Human TSLP ELISA kit
(DDXK-E-TSLP)

Intended use
The human TSLP ELISA kit is for quantitative determination of TSLP concentrations in human cell culture supernatants and biological fluids (plasma, serum). This kit is for research purpose only.

Background
TSLP (thymic stromal lymphopoïetin) is a hemopoietic protein of 159 aa (18 kDa), produced mainly by non-hematopoietic cells such as fibroblasts, epithelial cells and different types of stromal or stromal-like cells. It is known to play an important role in the maturation of T cell populations through activation of antigen presenting cells. It is also detected in the thymus and the tissue cells of the bone marrow. TSLP affects the transition from pre-B cells in B. Alternative splicing of this gene results in two transcripts variants. TSLP is proposed to signal through a heterodimeric receptor complex composed of TSLP-receptor and IL7R chain. Expressed in many tissues, it prevents apoptosis and stimulates the growth of myeloid cells. *(Reche PA et al, J Immunol., 2001, 167(1):336-43; Dorshkind. K, Nature. Immunol., 2000 (1):369-370).* Monoclonal antibodies raised against human TSLP were obtained after mice immunization with TSLP-His-tagged transfected 293T cells, and were used to set up a quantitative immunoassay to determine TSLP concentration in biological samples.

Kit contents
Capture Antibody: 0.5mg/mL of mouse anti-TSLP monoclonal antibody (clone 209A2.03). Dilute in carbonate buffer pH9.6 to a working concentration of 2.5µg/mL.
Detection Antibody: 0.5mg/mL of Biotin-conjugated anti-TSLP mouse monoclonal antibody (clone 210H1.04). Dilute to a working concentration of 3µg/mL in PBS-1% BSA-0.05% Tween20.
Standard: Each vial contains 1µg/mL of recombinant TSLP produced, purified and concentrated from eukaryotic cells. A 7-point standard curve using 2-fold serial dilutions in sample dilution buffer, and a high standard of 20 ng /mL is recommended.

Storage
All the reagents should be aliquoted before storage. Minimize repeated freeze and thaw. Refer to expiration date on all reagents prior to use. Do not mix reagents from different kits unless they have the same lot number.
-Standard storage: -80°C
-Capture and detection antibodies storage: -20°C

Materials and reagents required but not provided
96well-plate Nunc Maxi Sorp
50mM carbonate/bicarbonate buffer pH9.6
PBS-1% BSA-0.05% Tween20
Streptavidin-peroxydase concentrate (#SA-5004, Vector laboratories)
TMB super sensitive HRP (TMBS100-0500, TEBU-BIO)
Multichannel pipettes and pipette tips
A standard microplate reader (620nm)

Sensitivity
The minimum detectable dose of human TSLP was estimated at 1ng/ml.

Principle of the assay

The human TSLP ELISA kit is for the quantitative determination of human TSLP in human cell culture supernatants, plasma, serum, and various biological fluids.

This ELISA kit contains the specific components required for the development of human TSLP sandwich ELISAs. Each kit contains sufficient materials to run ELISAs on 3 X 96-well plates.

The DDX TSLP ELISA kit is a solid phase sandwich ELISA (Enzyme-Linked Immunosorbent Assay). The capture monoclonal antibody specific for TSLP is coated on a 96-well plate.
Standards and samples are added to the wells, and any TSLP present binds to the immobilized antibody.
The wells are washed and a biotin-conjugated anti-TSLP monoclonal antibody is then added, producing an antibody-antigen-antibody "sandwich".
To produce color in proportion to the amount of TSLP present in the sample, Streptavidin-peroxydase followed by TMB substrate solution is loaded and absorbance is measured at 620 nm.

TSLP Elisa protocol

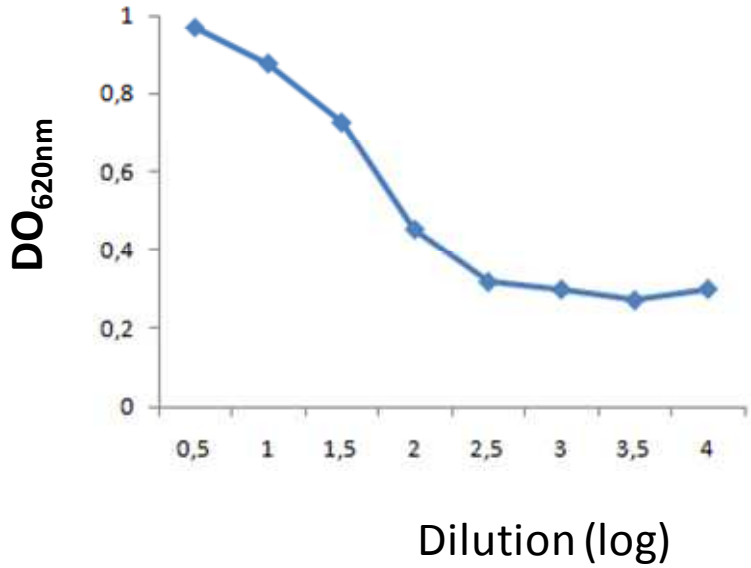
Pre-warm all the reagents to room temperature prior to setting up the assay

1. Coat the capture antibody at 2.5µg/ml, 120µl/well in Elisa coating buffer (50mM carbonate/bicarbonate buffer pH9.55-9.65). Seal the plate and incubate overnight at room temperature
2. Wash with 200µl PBS-0.05% Tween-20 at RT
3. Add 100µl/well of samples or standards diluted in PBS-1% BSA-0.05% Tween20
4. Incubate 2h at 37°C
5. Wash with 200µl PBS-0.05% Tween20 at RT
6. Add 100µl/well of biotin-conjugated detection antibody diluted at 3µg/ml in PBS-1% BSA-0.05% Tween20
7. Incubate 1h at 37°c
8. Wash 3 times with 200µl/well in PBS-0.05% Tween20
9. Add 100µl/well Streptavidin-peroxydase diluted at 1/2000 in PBS-1% BSA-0.05% Tween20
10. Incubate 30min at 37°C
11. Add 100µl/well TMB Super Sensitive HRP microwell substrate (Tebu Bio Laboratories)
12. Determine the optical density of each well using a microplate reader set at 620nm.

Standard curve

Each laboratory should establish its own standard curve.

Here is an example of standard curve using serial dilutions of TSLP (20ng/ml) prepared from supernatants of TSLP-transfected eukaryotic cells.



Troubleshootings

To obtain good and reproducible results, usage of sterile reagents and clean materials is strongly recommended. All basic reagents such as washing and dilution buffers, water, must be devoid of contamination.

To ensure pH stability, incubation at 37°C should be performed in a humidified atmosphere of 5% CO_2

Problems	Possible Sources	Solutions
Poor detection value	The concentration of antigen in samples was too low	Enriching samples to increase the concentration of antigen
	Samples were ineffective	Check if the samples are stored at cold environment. Detect samples in timely manner
No signal	Incorrect or no Detection Antibody was added	Add appropriate Detection Antibody and continue
	Substrate solution was not added	Add substrate solution and continue
	Incorrect storage condition	Check if the kit is stored at recommended condition and used before expiration date
Poor Standard Curve	Inappropriate storage	Aliquot standard and store at -70°C
	Imprecise / inaccurate pipetting	Check / calibrate pipettes
	Incubations done at inappropriate temperature or timing	Follow the general ELISA protocol
	Background wells were contaminated	Avoid cross contamination by using the sealer appropriately
High Background	Insufficient washes	Use multichannel pipettes without touching the reagents on the plate
		Increase cycles of washes and soaking time between washes
	TMB Substrate Solution was contaminated	TMB Substrate Solution should be clear and colorless prior to addition to wells
	Materials were contaminated	Use clean plates, tubes and pipettes tips
Non-specificity	Samples were contaminated	Avoid cross contamination of samples
	The concentration of samples was too high	Try higher dilution rate of samples