Quantikine™ ELISA

Human TSLP Immunoassay

Catalog Number DTSLP0

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

Note: For accurate sample detection, serum samples may require a 1:2 dilution.

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 R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
TEL: 800 343 7475 612 379 2956
FAX: 612 656 4400
E-MAIL: info@bio-techne.com

Distributed by:

Europe | Middle East | Africa Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info.emea@bio-techne.com

China Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
TEL: +86 (21) 52380373 (400) 821-3475
FAX: +86 (21) 52371001
E-MAIL: info.cn@bio-techne.com

INTRODUCTION

Thymic Stromal Lymphopoietin (TSLP) is a 23 kDa member of the IL-7 family of α-helical cytokines (1-3). It is a monomeric glycoprotein that is synthesized as a 159 amino acid (aa) precursor (4, 5). 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 (6). 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 (5, 7). In the mature region, human TSLP shares 37% aa sequence identity with mouse TSLP (5).

A high affinity receptor for TSLP has been reported. It is composed of two subunits: a 48 kDa low-affinity TSLP receptor and a 70 kDa IL-7 receptor alpha (IL-7 Rα) (1, 8, 9). 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 (8). 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 (1, 3, 8). It binds TSLP with low affinity and does not bind IL-7 at all (10). When complexed to IL-7 Rα, TSLP R activates STAT5 but not Jaks (7). It is proposed that Tec protein kinases substitute for the Jak kinase system (3, 4).

TSLP appears to have species-specific functions. In mouse, TSLP was initially reported to act on NK cells, mast cells, and B cells, but this does not appear to occur in humans. In humans, TSLP is produced by a number of divergent cell types, all of which appear to target T cells, monocytes, and/or dendritic cells (2). On TCR-activated T cells, TSLP directly induces T cell proliferation (11). The significance of this direct action is unclear. On monocytes, TSLP is reported to induce the release of multiple chemokines that target CCR4, a receptor associated with the Th2 subset (5). TSLP is best known for its direct action on subsets of dendritic cells. In thymic medulla, Hassell's corpuscle epithelium produces TSLP that acts on resident CD11c+ dendritic cells. This induces the expression of B7 family molecules on dendritic cells, which subsequently convert regional $CD4^+CD25^+$ (potentially) autoreactive T cells into $CD4^+CD25^+FOXP3^+$ regulatory T cells (12). Allergen-challenged keratinocytes are also known to produce TSLP in skin where TSLP acts on Langerhans cells (CD1a+ immature dendritic cells) which then migrate to regional lymph nodes and express B7-2/CD86, CD83, high levels of MHC-II, and TARC. TARC attracts naïve $CD4^+$ T cells to the TSLP-activated Langerhans cells, and this interaction induces a Th2 phenotype. TSLP-induced Th2 cells are strong producers of IL-13, IL-5, and TNF-α, all of which promote allergic-type inflammations (13, 14).

The Quantikine™ Human TSLP immunoassay is a 4.5 hour sandwich-type solid phase ELISA designed to measure human TSLP in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human TSLP and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human 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 naturally occurring human TSLP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for human TSLP has been pre-coated onto a microplate. Standards 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 polyclonal antibody specific for human TSLP is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of TSLP bound in the initial step. The color development is stopped and the intensity of the color is measured.

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.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
Human TSLP Microplate	893574	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for human 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.*
Human TSLP Conjugate	893575	21 mL of a polyclonal antibody specific for human TSLP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human TSLP Standard	893576	Recombinant human TSLP in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives. *May contain crystals. Warm to room temperature and mix well before and during use.*	
Calibrator Diluent RD6-10	895468	21 mL of a buffered protein base 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	895032	6 mL of 2 N sulfuric 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
- **Polypropylene** test tubes for dilution of standards
- Human TSLP Controls (optional; R&D Systems®, Catalog # QC178)

PRECAUTIONS

Calibrator Diluent RD6-10 contains 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 SDS 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 and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Serum - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 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 15 minutes at 1000 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.*
Hemolyzed samples are not suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 50 µL of sample + 50 µL of Calibrator Diluent RD6-10.

REAGENT PREPARATION

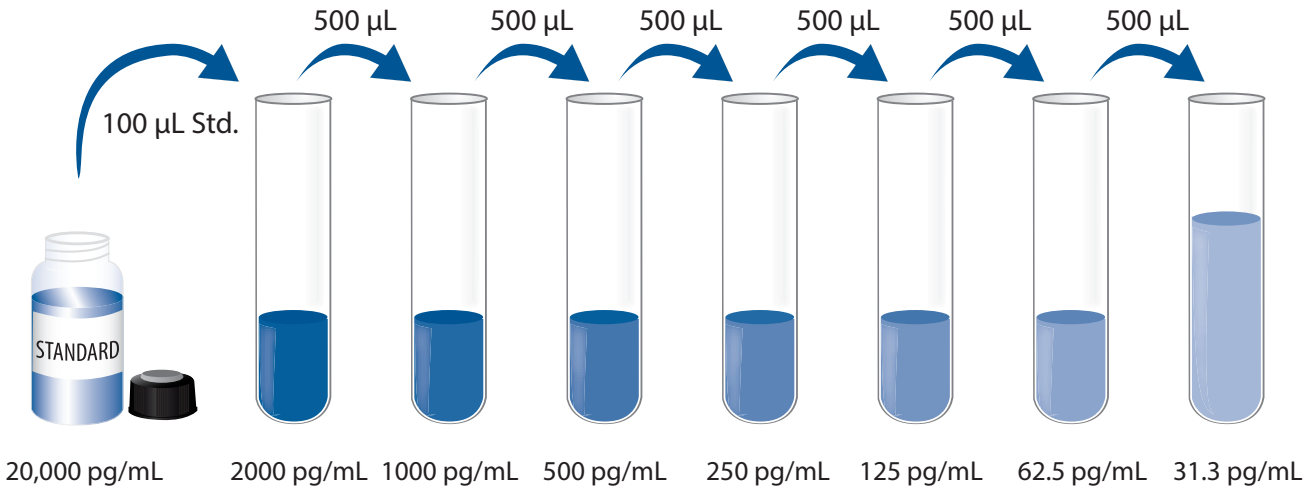
Bring all reagents to room temperature before use.

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 480 mL of 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. 200 µL of the resultant mixture is required per well.

Human TSLP Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human TSLP Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD6-10 into the 2000 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. The Calibrator Diluent 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, controls, and samples be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1X to each well. *Assay Diluent RD1X may contain crystals. Mix well before and during use.*
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature. A plate layout is provided to record standards and samples assayed.
5. Aspirate each well and wash, repeating the process three times for a total of four 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 200 µL of Human 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 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, 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

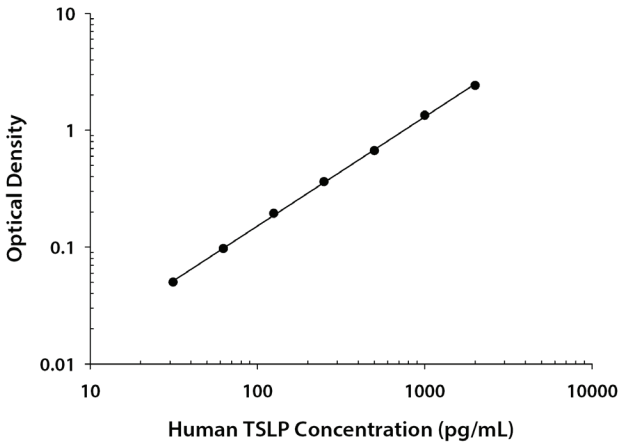
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a log/log 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 a log/log graph. The data may be linearized by plotting the log of the human TSLP concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

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	0.017 0.019	0.018	—
31.3	0.067 0.069	0.068	0.050
62.5	0.114 0.115	0.115	0.097
125	0.210 0.214	0.212	0.194
250	0.378 0.382	0.380	0.362
500	0.677 0.700	0.689	0.671
1000	1.345 1.374	1.360	1.342
2000	2.416 2.450	2.433	2.415

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 forty 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	40	40	40
Mean (pg/mL)	193	729	1318	230	775	1341
Standard deviation	17.9	52.8	108	21.9	53.4	80.6
CV (%)	9.3	7.2	8.2	9.5	6.9	6.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	99	90-107%
Serum (n=4)	94	88-101%
EDTA plasma (n=4)	96	81-105%
Heparin plasma (n=4)	97	91-104%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human TSLP were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	109	110	104	106
	Range (%)	106-115	103-118	100-107	101-112
1:4	Average % of Expected	105	112	112	109
	Range (%)	99-107	109-116	104-120	101-115
1:8	Average % of Expected	102	109	108	104
	Range (%)	100-103	104-116	107-111	96-110
1:16	Average % of Expected	100	104	107	101
	Range (%)	90-108	97-113	100-116	93-106

SENSITIVITY

Fifty-two assays were evaluated and the minimum detectable dose (MDD) of human TSLP ranged from 1.05-9.87 pg/mL. The mean MDD was 3.46 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 *E. coli*-expressed recombinant human TSLP produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Seventy-one samples from apparently healthy volunteers were evaluated for the presence of human TSLP in this assay. Only one donor had detectable levels of TSLP. The serum, EDTA plasma, and heparin plasma samples from this donor averaged 259 pg/mL. All other samples read below the low standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood lymphocytes (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of natural human TSLP. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human 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 recombinant human TSLP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ANG
Ang-2
Amphiregulin
BDNF
CD4
CD40
CD40 Ligand
CNTF
Cardiotrophin-1
CTLA-4
Epo
Fas
GDNF
GITR
GITR Ligand
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
IL-9
IL-10
IL-11
IL-12
IL-12/IL-23 p40
IL-13
IL-15
IL-16
IL-17
IL-19
IL-20
IL-22
IL-24
IL-26 (dimer)
IL-26 (monomer)
IL-28A
IL-29
Leptin
LIF
MIF
MK
NT-3
NT-4
OPG
OSM
PTN
SCF
SLPI
SMDF
TNF-α
TNF-β
Tpo
TRAIL
TRANCE

Recombinant mouse:

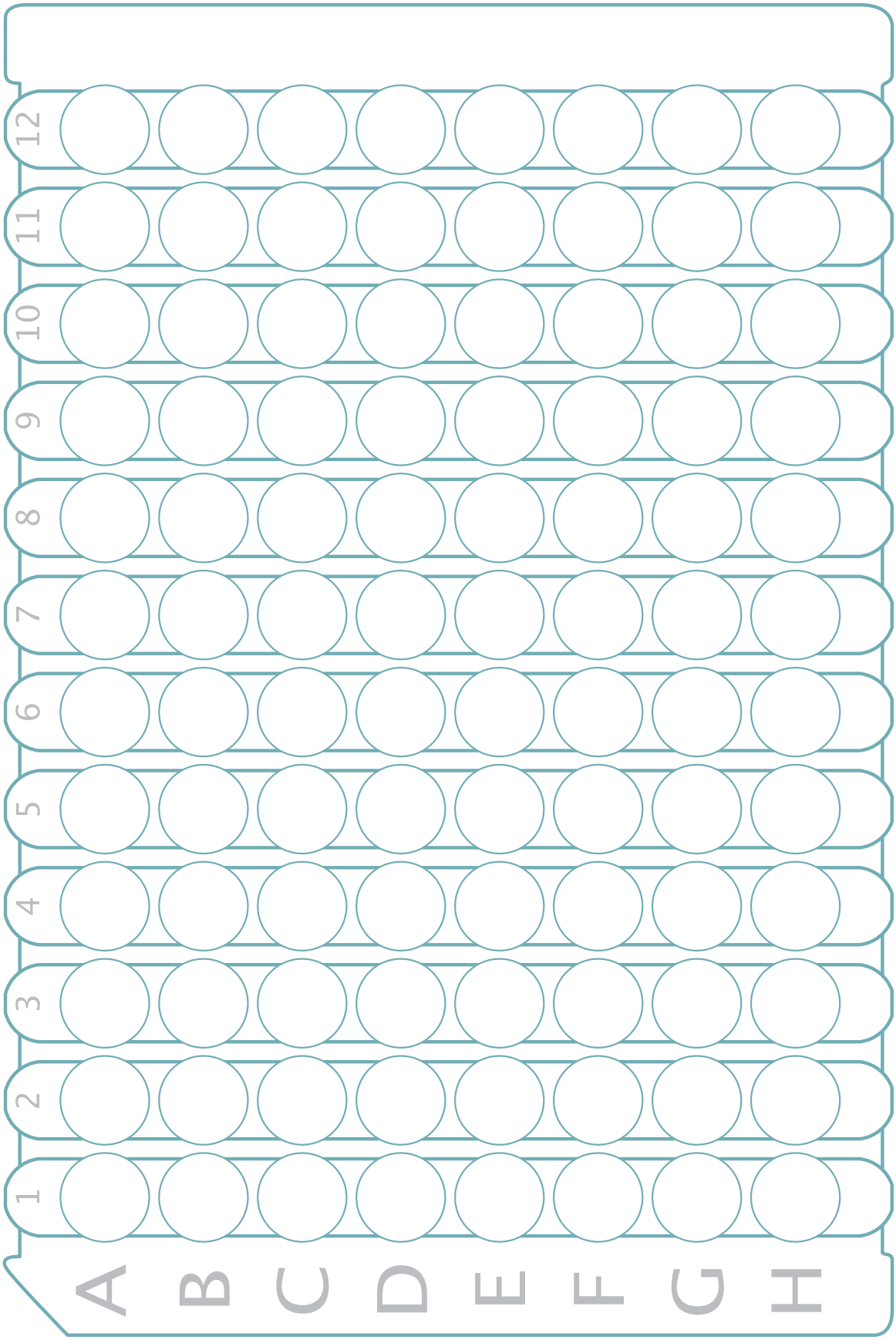
Cardiotrophin-1
CTLA-4
Fas
Fas Ligand
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-11
IL-12
IL-12/IL-23 p40
IL-13
IL-17
Leptin
LIF
OPG
OPN
OSM
SCF
TNF-α
Tpo
TRANCE
TSLP

REFERENCES

1. Liu, Y-J. *et al.* (2007) Annu. Rev. Immunol. **25**:193.
2. Liu, Y-J. (2006) J. Exp. Med. **203**:269.
3. Ziegler, S.F. and Y-J. Liu (2006) Nat. Immunol. **7**:709.
4. Quentmeier, H. *et al.* (2001) Leukemia **15**:1286.
5. Reche, P.A. *et al.* (2001) J. Immunol. **167**:336.
6. GenBank Accession #: EAW49038.
7. Sims, J.E. *et al.* (2000) J. Exp. Med. **192**:671.
8. Zhang, W. *et al.* (2001) Biochem. Biophys. Res. Commun. **281**:878.
9. Goodwin, R.G. *et al.* (1990) Cell **60**:941.
10. Pandey, A. *et al.* (2000) Nat. Immunol. **1**:59.
11. Rochman, I. *et al.* (2007) J. Immunol. **178**:6720.
12. Watanabe, N. *et al.* (2005) Nature **436**:1181.
13. Ebner, S. *et al.* (2007) J. Clin. Allergy Immunol. **119**:982.
14. Soumelis, V. *et al.* (2002) Nat. Immunol. **3**:673.

PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES

NOTES