Quantikine® ELISA

Human CXCL11/I-TAC Immunoassay

Catalog Number DCX110

For the quantitative determination of human Interferon-inducible T cell alpha Chemoattractant (I-TAC) concentrations in cell culture supernates, serum, and plasma.

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

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INTRODUCTION

Interferon-inducible T cell Alpha Chemoattractant (I-TAC; also known as CXCL11) is a non-ELR (lacking the Glu-Leu-Arg tripeptide motif) CXC chemokine. The cDNA encoding I-TAC (1), originally designated β-R1 (2), has also been reported as SCYB9B (3), H174 (4), IP-9 (5), and SCYB11 (6). I-TAC expression is significantly increased in response to IFN-β or IFN-γ, and is presumed to be involved in pathologies characterized by the presence of activated T cells.

The I-TAC gene consists of four exons interrupted by three introns of 585, 98, and 230 base pairs (7). I-TAC cDNA encodes a 94 amino acid (aa) residue precursor protein with a 21 aa residue putative signal sequence that is cleaved to generate the mature protein (1). I-TAC is 36% and 37% identical in primary structure to the other non-ELR CXC chemokines IP-10 and MIG, respectively (1). Mouse I-TAC exhibits 68% aa sequence homology with human I-TAC (8). In humans, I-TAC, IP-10 and MIG all map to the same locus on chromosome 4 (1, 3, 9).

I-TAC exclusively utilizes CXC chemokine receptor 3 (CXCR3) (1, 10), a G protein-coupled receptor expressed primarily on activated T cells, yet also found on endothelial cells (11). Among activated T cells, CXCR3 is more highly expressed on the Th1 subset (12). IP-10 and MIG also bind CXCR3, but with lower affinity (1, 10) and less potency (1) than I-TAC. Furthermore, there is evidence that I-TAC is able to bind both the free and coupled forms of the receptor, whereas IP-10 and MIG bind only the coupled receptor (10).

The I-TAC promoter region contains the consensus sequences for the interferon-responsive DNA regulatory elements ISRE, GAS, and the cytokine-responsive binding protein NF-κB (7). I-TAC expression is strongly upregulated in response to IFN-γ. This effect is dramatically enhanced by addition of IL-1β (1) or TNF-α (7), though these pro-inflammatory cytokines alone fail to induce expression. I-TAC mRNA can be induced in astrocytes and monocytes (1), bronchial epithelial cells (13), intestinal epithelial cells (14), endothelial cells (15, 16), keratinocytes (5, 17), macrophages (15), and neutrophils (18). I-TAC expression is suppressed by the Th2 cytokines IL-4 and IL-10 (18) and also by peroxisome proliferator-activated receptor γ activators (19). I-TAC recruits activated Th1 lymphocytes to sites of inflammation. It is an antagonist of CC chemokine receptor 3 (CCR3) (20), a chemokine receptor expressed on eosinophils and Th2 lymphocytes, suggesting that I-TAC may act to further polarize T cell recruitment at sites of expression. There is evidence that I-TAC may be influential in the migration of different subsets of mature thymocytes during thymus lymphopoiesis (21). I-TAC also exhibits an angiostatic effect, apparently via CXCR3 expressed on endothelial cells in the S/G2-M phase of the cell cycle (11). I-TAC has been implicated in allergic contact dermatitis (22), atherosclerosis (15), mycosis fungoides (a type of T cell lymphoma) (23), and immune-mediated disorders of the central nervous system such as multiple sclerosis (24).

The Quantikine Human CXCL11/I-TAC Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human I-TAC in cell culture supernates, serum, and plasma. It contains *E. coli*- expressed recombinant human I-TAC and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human I-TAC 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 I-TAC.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human I-TAC has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any I-TAC present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human I-TAC 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 I-TAC 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 the appropriate Calibrator Diluent and repeat the assay.
- Any variation in standard 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 I-TAC Microplate	891030	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human I-TAC.	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 I-TAC Conjugate	891031	21 mL of polyclonal antibody specific for human I-TAC conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human I-TAC Standard	891032	Recombinant human I-TAC in a buffer with preservative; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-68	895528	11 mL of a buffer with preservative.	
Calibrator Diluent RD5-21	895348	21 mL of a buffered protein base with preservative. *For cell culture samples.*	
Calibrator Diluent RD6P	895118	21 mL of animal serum with preservative. *For serum/plasma samples.*	
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	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 I-TAC Controls (optional; R&D Systems, Catalog # QC23).

PRECAUTIONS

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. Please 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 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.*
Grossly hemolyzed samples are not suitable for use in this assay.
Heparin appears to release erythrocyte-bound I-TAC, resulting in higher measured concentrations than matched EDTA plasma or serum samples. See the Sample Values section.

REAGENT PREPARATION

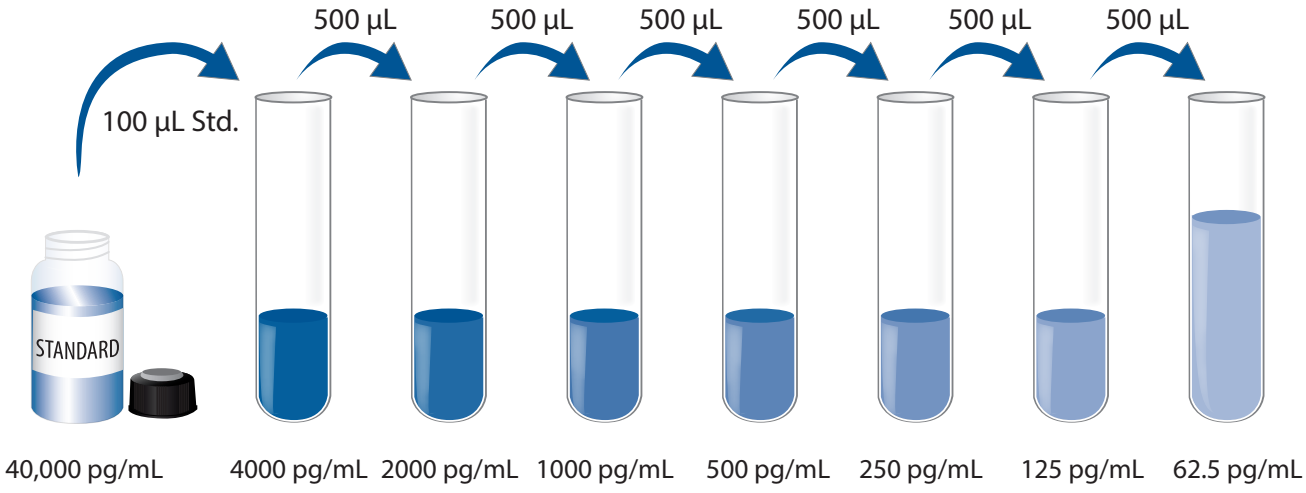
Bring all reagents to room temperature before use.

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 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 I-TAC Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human I-TAC Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 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 RD5-21 (*for cell culture supernate samples*) or Calibrator Diluent RD6P (*for serum/plasma samples*) into the 4000 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 4000 pg/mL standard serves as the high standard. The appropriate 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, samples, and controls 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 RD1-68 to each well.
4. Add 100 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 I-TAC 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.).

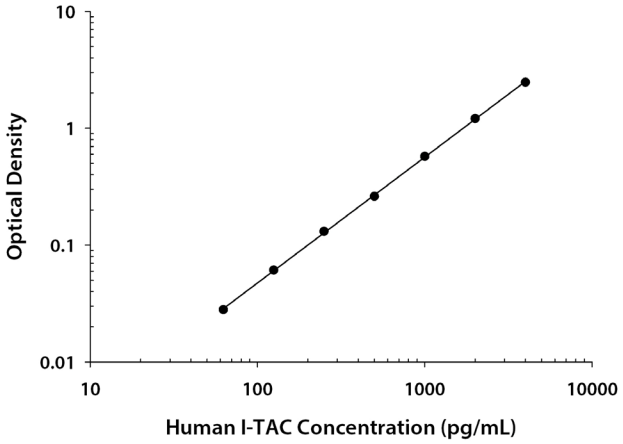
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human I-TAC 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

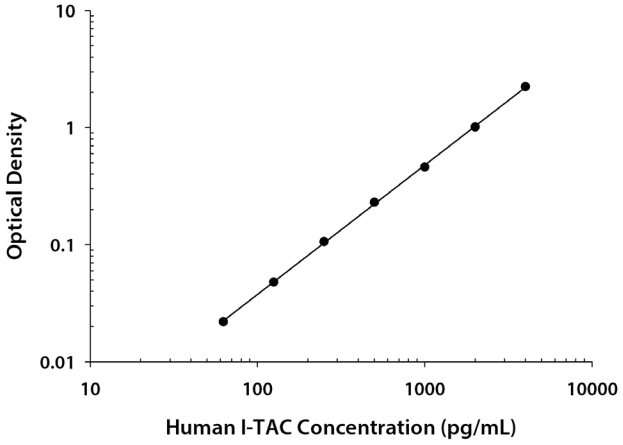
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.053 0.057	0.055	—
62.5	0.079 0.086	0.083	0.028
125	0.114 0.118	0.116	0.061
250	0.178 0.194	0.186	0.131
500	0.309 0.322	0.316	0.261
1000	0.622 0.634	0.628	0.573
2000	1.261 1.262	1.262	1.207
4000	2.459 2.587	2.523	2.468

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.018 0.019	0.019	—
62.5	0.040 0.042	0.041	0.022
125	0.067 0.067	0.067	0.048
250	0.124 0.125	0.125	0.106
500	0.246 0.251	0.249	0.230
1000	0.474 0.485	0.480	0.461
2000	1.019 1.038	1.029	1.010
4000	2.195 2.312	2.254	2.235

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.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	353	1179	2191	409	1290	2401
Standard deviation	18.0	40.6	103	35.5	93.0	179
CV (%)	5.1	3.4	4.7	8.7	7.2	7.5

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	403	1321	2481	437	1403	2644
Standard deviation	19.0	62.5	147	35.1	107	190
CV (%)	4.7	4.7	5.9	8.0	7.6	7.2

RECOVERY

The recovery of human I-TAC spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	91-111%
Serum (n=5)	106	93-117%
EDTA plasma (n=5)	98	85-112%
Heparin plasma (n=5)	98	90-106%

LINEARITY

To assess linearity of the assay, samples containing and/or spiked with high concentrations of human I-TAC were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	103	106	105	103
	Range (%)	96-109	97-111	99-108	100-107
1:4	Average % of Expected	105	103	107	106
	Range (%)	99-113	93-108	98-115	102-109
1:8	Average % of Expected	106	99	106	107
	Range (%)	96-111	90-104	103-111	102-111
1:16	Average % of Expected	105	95	104	99
	Range (%)	98-111	89-100	101-112	96-102

SENSITIVITY

Fifty-six assays were evaluated and the minimum detectable dose (MDD) of human I-TAC ranged from 3.4-39.7 pg/mL. The mean MDD was 13.9 pg/mL.

The MDD was determined by adding two standard deviations to the mean optical density 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 I-TAC produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human I-TAC in this assay. No medical histories were available for the donors used in this study.

Note: *Heparin appears to release erythrocyte-bound I-TAC, resulting in higher measured concentrations than matched EDTA plasma or serum samples.*

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=35)	177	17	ND-290
EDTA plasma (n=35)	136	26	ND-269
Heparin plasma (n=60)	440	100	100-1208

ND=Non-detectable

Cell Culture Supernates:
Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed and assayed for levels of human I-TAC.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND
Stimulated	1655	1088

ND=Non-detectable

THP-1 human acute monocytic leukemia cells (5 x 10^5 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate at 37 °C and 5% CO_2. Cells were stimulated with 1 µg/mL of recombinant human IFN-γ for 8 hours, 1 µg/mL of LPS was added, and the cells were incubated for an additional 8 hours. An aliquot of the cell culture supernate was removed, assayed for human I-TAC, and measured 2348 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human I-TAC.

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 I-TAC control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

ENA-78
BLC/BCA-1
GCP-2
GROα
GROβ
GROγ
IFN-γ
IL-8
IL-8, endothelial cell-derived
IP-10
MIG
NAP-2
SDF-1α
SDF-1β

Recombinant mouse:

BLC/BCA-1
GCP-2
IP-10/CRG-2
I-TAC
KC
MIG
SDF-1α

Recombinant porcine:

IL-8

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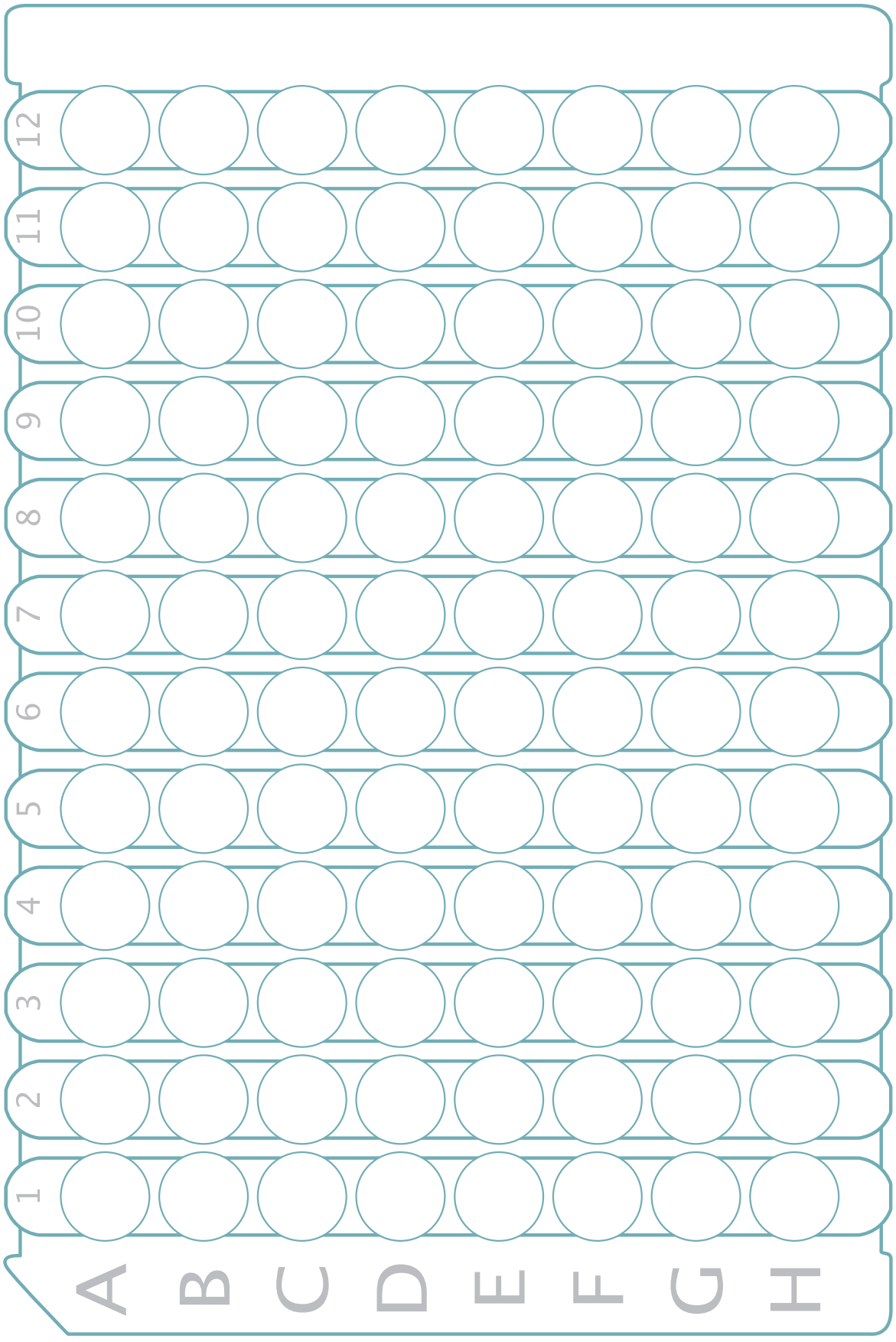
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES