Quantikine® ELISA

Human IL-13 Immunoassay

Catalog Number D1300B
S1300B
PD1300B

For the quantitative determination of human Interleukin 13 (IL-13) 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

Interleukin-13 (IL-13) is a 17 kDa immunoregulatory cytokine that plays a key role in the pathogenesis of allergy, cancer, and tissue fibrosis (1-3). It is secreted by Th1, Th2, Th17, NK, and mast cells, visceral smooth muscle cells, eosinophils, and basophils (2, 4). This pattern is similar to the expression of Interleukin-4 (IL-4) but also includes subsets of Th1 and Th17 cells that do not secrete IL-4 (5). IL-13 circulates as a monomer and has two internal disulfide bonds that contribute to its bundled four α-helix configuration (6, 7). Mature human IL-13 shares approximately 58% amino acid sequence identity with mouse and rat IL-13. Despite the low homology, it exhibits cross-species activity between human, mouse, and rat (8, 9). IL-13 suppresses the production of proinflammatory cytokines and other cytotoxic substances by macrophages, fibroblasts, and endothelial cells. On B cells, it promotes cellular activation, immunoglobulin class switching to IgE, and the upregulation of CD23/Fcε RII. Polymorphisms and upregulation of IL-13 are associated with atopy, asthma, airway hyperresponsiveness, and tissue fibrosis (1, 10).

The biological effects of IL-13 and IL-4 are closely related due in part to a shared receptor system. IL-13 binds with low affinity to the transmembrane IL-13 Rα1 which then forms a signaling complex with the transmembrane IL-4 Rα (11-13). This high affinity receptor complex also functions as the type 2 IL-4 receptor (11, 12). Soluble forms of IL-4 Rα are expressed which retain ligand binding properties and inhibit IL-4 bioactivity (14, 15). IL-4 Rα also associates with the common gamma chain (γc) to form the type 1 IL-4 receptor complex (16, 17).

Additionally, IL-13 binds with high affinity to IL-13 Rα2 which is expressed as cell surface and soluble forms (18-20). IL-13 Rα2 functions as a decoy receptor by preventing IL-13 from signaling through the IL-13 Rα1/IL-4 Rα complex (21, 22). It also inhibits responsiveness to IL-4 by blocking signaling through IL-4-occupied IL-13 Rα1/IL-4 Rα receptor complexes (22, 23). IL-13 Rα2 is upregulated during Th2-biased immune responses and limits inflammatory tissue damage (20, 24, 25). Aside from its decoy function, IL-13 Rα2 can signal in response to IL-13 to directly promote tumor cell invasiveness and the development of tissue fibrosis (26-28).

The Quantikine® Human IL-13 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-13 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human IL-13 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant human IL-13 accurately. Results obtained using natural human IL-13 showed dose response 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 human IL-13.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-13 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-13 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-13 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 IL-13 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.
- It is important that the calibrator diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate 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 #	CATALOG # D1300B	CATALOG # S1300B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IL-13 Microplate	894379	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-13.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IL-13 Conjugate	894380	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human IL-13 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-13 Standard	894381	1 vial	6 vials	Recombinant human IL-13 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5R	895190	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-64	895995	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

D1300B contains sufficient materials to run an ELISA on one 96 well plate.
S1300B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PD1300B). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- Human IL-13 Controls (optional; R&D Systems®, Catalog # QC20).

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. 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.*

REAGENT PREPARATION

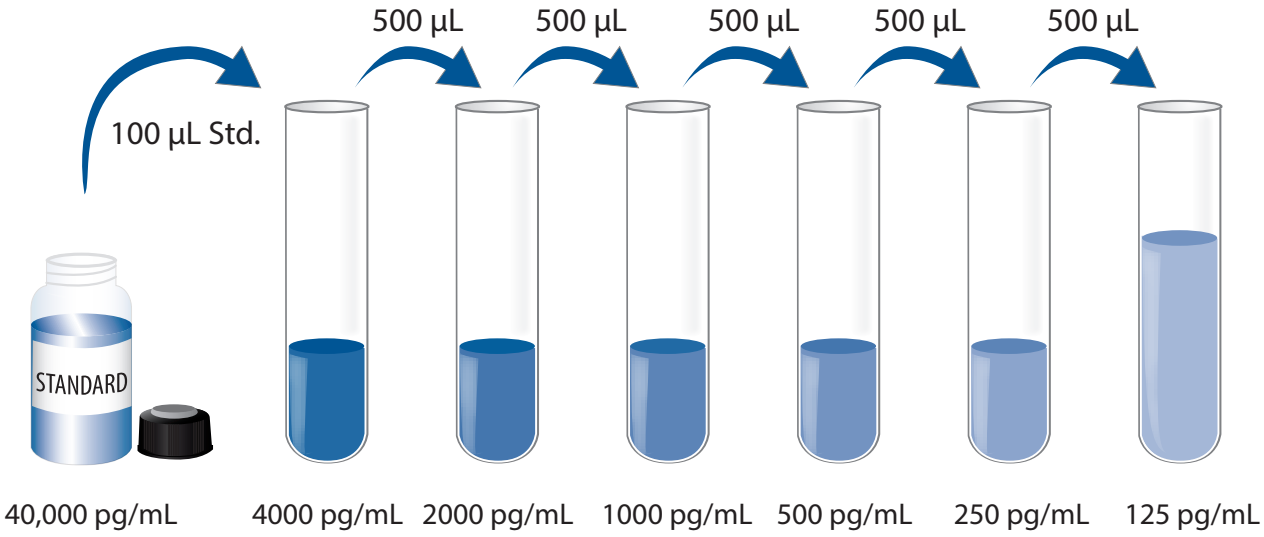
Bring all reagents to room temperature before use.

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 IL-13 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-13 Standard with deionized or distilled water. This reconstitution produces a stock solution of 40,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-64 (*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 dilution 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, samples, and working standards 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 RD1W per 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 IL-13 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 if 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.).

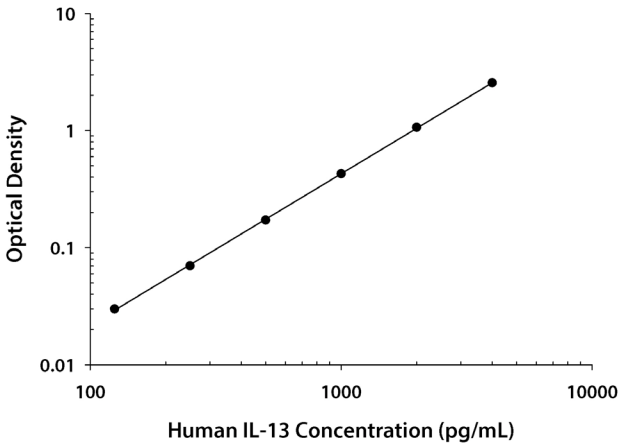
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human IL-13 concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If the samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

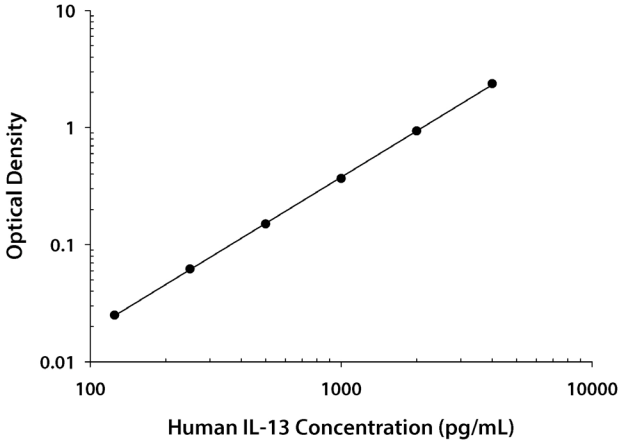
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.007 0.008	0.008	—
125	0.037 0.038	0.038	0.030
250	0.076 0.079	0.078	0.070
500	0.177 0.182	0.180	0.172
1000	0.433 0.438	0.436	0.428
2000	1.042 1.104	1.073	1.065
4000	2.505 2.622	2.564	2.556

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.009 0.010	0.010	—
125	0.034 0.036	0.035	0.025
250	0.070 0.073	0.072	0.062
500	0.159 0.160	0.160	0.150
1000	0.375 0.378	0.377	0.367
2000	0.940 0.949	0.945	0.935
4000	2.377 2.379	2.378	2.368

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 twenty 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	20	20	20
Mean (pg/mL)	360	1031	2002	319	932	1895
Standard deviation	12.9	27.5	43.2	22.2	52.6	108
CV (%)	3.6	2.7	2.2	7.0	5.6	5.7

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	352	960	1919	365	1087	2199
Standard deviation	11.1	21.3	33.3	25.8	63.1	105
CV (%)	3.2	2.2	1.7	7.1	5.8	4.8

SENSITIVITY

Sixty-three assays were evaluated and the minimum detectable dose (MDD) of human IL-13 ranged from 3.46-57.4 pg/mL. The mean MDD was 13.2 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 IL-13 produced at R&D Systems®.

The NIBSC/WHO International Standard (94/622) was evaluated in this kit. To convert sample values obtained with the Quantikine® Human IL-13 kit to approximate NIBSC/WHO 94/622 Units, use the equation below.

NIBSC/WHO (94/622) approximate value (U/mL) = 0.0015 x Quantikine® Human IL-13 value (pg/mL)

RECOVERY

The recovery of human IL-13 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)	108	100-120%
Serum (n=4)	102	95-113%
EDTA plasma (n=4)	106	98-120%
Heparin plasma (n=4)	104	94-120%

LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of human IL-13 and diluted with the appropriate 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	97	98	95	93
	Range (%)	92-104	92-101	89-99	81-101
1:4	Average % of Expected	95	95	94	90
	Range (%)	91-103	91-98	89-99	77-97
1:8	Average % of Expected	92	98	94	95
	Range (%)	87-96	92-101	90-96	82-103
1:16	Average % of Expected	95	103	96	97
	Range (%)	90-97	91-114	93-97	84-102

SAMPLE VALUES

Serum/Plasma - Thirty-five samples from apparently healthy volunteers were evaluated for the presence of human IL-13 in this assay. No medical histories were available for the donors used in this study. No detectable levels were observed.

Cell Culture Supernates - Human peripheral blood leukocytes 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. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 2 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-13.

Condition	Day 2 (pg/mL)	Day 5 (pg/mL)
Unstimulated, Sample 1	ND	ND
Stimulated, Sample 1	1565	2529
Unstimulated, Sample 2	ND	ND
Stimulated, Sample 2	846	1447

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IL-13.

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 human IL-13 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-4
IL-4I1
IL-4 Rα
IL-13 Rα1

Recombinant mouse:

IL-4
IL-4I1
IL-4 Rα
IL-13

Recombinant rat:

IL-4
IL-13

Recombinant canine:

IL-4
IL-13

Recombinant rhesus macaque:

IL-4
IL-13

Other recombinants:

bovine IL-4
cotton rat IL-4
equine IL-4
feline IL-4
porcine IL-4

Recombinant human IL-13 Rα2 interferes at concentrations ≥ 1.0 ng/mL.

Recombinant mouse IL-13 Rα1 interferes at concentrations ≥ 25 ng/mL.

Recombinant mouse IL-13 Rα2 interferes at concentrations ≥ 1.0 ng/mL.

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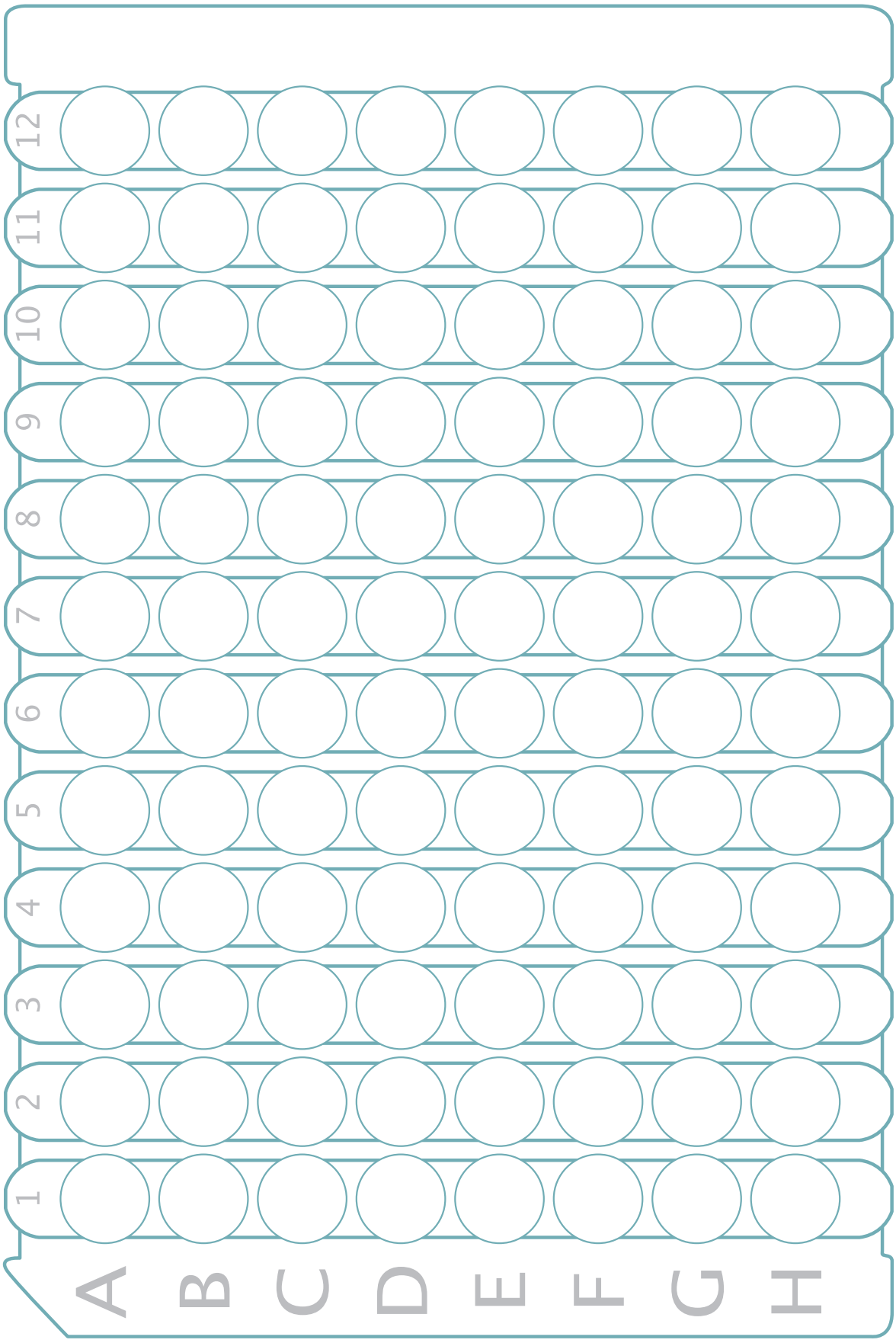
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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