Quantikine® ELISA

Human IL-19 Immunoassay

Catalog Number D1900

For the quantitative determination of human Interleukin 19 (IL-19) concentrations in cell culture supernates, serum, plasma, saliva, urine, and human milk.

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 19 (IL-19) is a member of the IL-10 family of class II α-helical cytokines that contains viral and cellular homolog proteins (1-3). Mature human IL-19 is secreted as a 35-40 kDa N-glycosylated monomer (3, 4). It shares 69% amino acid (aa) sequence identity with mouse and rat IL-19. Under normal conditions, IL-19 expression is primarily restricted to monocytes. Upon inflammatory stimulation, it is upregulated in monocytes as well as in keratinocytes, smooth muscle cells, and airway epithelial cells (3, 5-8). IL-19 induces the release of inflammatory mediators and KGF from monocytes, dendritic cells, lung epithelial cells, hepatocytes, or $CD8^+$ T cells (9-12). It drives T-helper cell differentiation towards a Th2 response, inducing both IL-10 and additional production of itself (10, 13, 14).

IL-19 exerts its effects through a heterodimeric complex composed of the transmembrane class II cytokine receptors IL-20 Rα and IL-20 Rβ (1, 15, 16). It binds to IL-20 Rβ which then associates with IL-20 Rα to form a signaling receptor complex (17, 18). The two receptor subunits do not interact in the absence of ligand (17). This receptor complex also mediates IL-20 and IL-24 effects (15-19). In addition, IL-20 Rα heterodimerizes with IL-10 Rβ to form the functional receptor complex for IL-26, and IL-20 Rβ heterodimerizes with IL-22 R to form the functional receptor complex for IL-20 and IL-24 (15, 18, 20). IL-20 Rα is expressed in skin, heart, placenta, salivary gland, testis, and prostate gland (15, 16). Strong IL-20 Rβ expression is normally restricted to skin, testis, and prostate (15, 16). The expression of both IL-20 Rα and IL-20 Rβ are upregulated in psoriatic skin lesions on keratinocytes, immune cells, and endothelial cells (16).

Serum IL-19 is elevated in asthma, uremia, and septic shock (11, 13, 21). It is produced within sites of inflammation such as airway epithelia in asthma, basal and suprabasal keratinocytes in psoriasis, and vascular smooth muscle cells following arterial injury (5, 7, 12, 22). IL-19 plays a protective role following balloon angioplasty but promotes neutrophil infiltration and tissue damage in mouse models of septic shock (7, 11).

The Quantikine® Human IL-19 immunoassay is a 4.5 hour solid phase ELISA designed to measure human IL-19 in cell culture supernates, serum, plasma, saliva, urine, and human milk. It contains NS0-expressed recombinant human IL-19 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IL-19 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 human IL-19.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-19 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-19 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IL-19 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-19 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 IL-19 Microplate	892688	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IL-19.	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 IL-19 Conjugate	892689	21 mL of a monoclonal antibody specific for human IL-19 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-19 Standard	892690	Recombinant human IL-19 in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	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.
- Test tubes for dilution of standards and samples.
- Human IL-19 Controls (optional; available from R&D Systems®, Catalog # QC24).

PRECAUTIONS

IL-19 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 heparin or EDTA 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.*

Saliva - Collect saliva in a tube, and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva values are decreased when a Salivette® or other collection device is used.*

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Human Milk - Centrifuge for 15 minutes at 1000 x g at 2-8 °C. Collect the aqueous fraction and centrifuge twice more for a total of 3 times. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Human milk samples require at least a 20-fold dilution. A suggested 20-fold dilution is 20 µL of sample + 380 µL of Calibrator Diluent RD5K.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

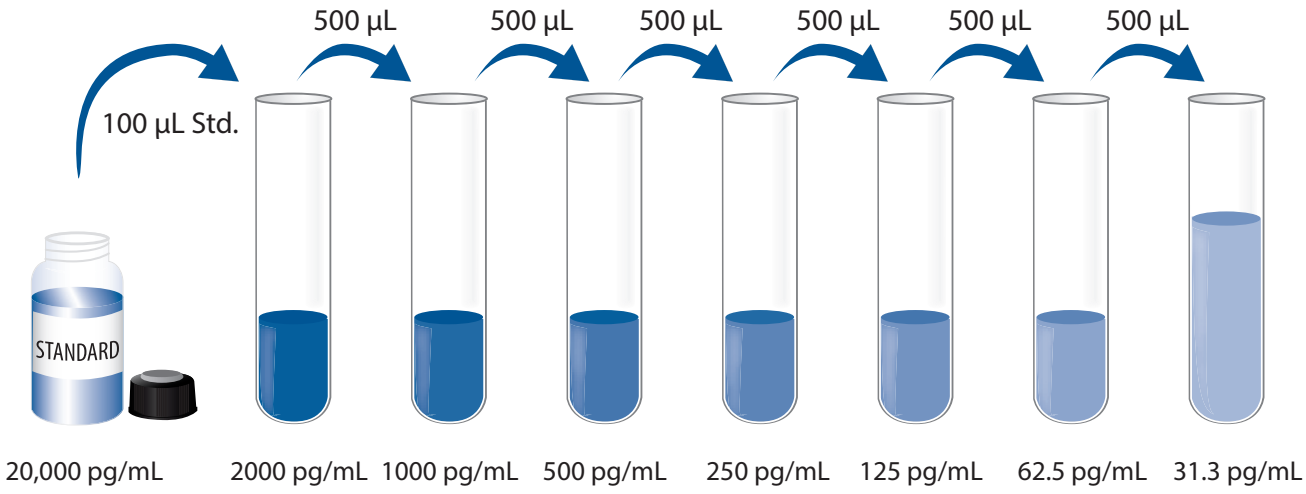
Note: *High concentrations of IL-19 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 IL-19 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IL-19 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.

Pipette 900 µL of Calibrator Diluent RD5K 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. Calibrator Diluent RD5K 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.

Note: *High concentrations of IL-19 are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 RD1-19 to each well.
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 IL-19 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

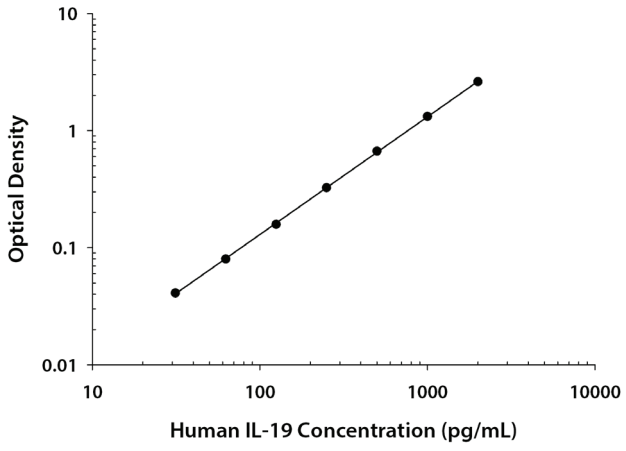
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-19 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

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	0.014 0.015	0.015	—
31.3	0.055 0.056	0.056	0.041
62.5	0.092 0.097	0.095	0.080
125	0.172 0.174	0.173	0.158
250	0.338 0.342	0.340	0.325
500	0.674 0.689	0.682	0.667
1000	1.298 1.370	1.334	1.319
2000	2.611 2.637	2.624	2.609

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)	292	805	1586	269	739	1478
Standard deviation	10.3	19.0	41.8	22.0	56.8	103
CV (%)	3.5	2.4	2.6	8.2	7.7	7.0

RECOVERY

The recovery of human IL-19 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	93-104%
Serum (n=4)	93	87-97%
EDTA plasma (n=4)	94	85-102%
Heparin plasma (n=4)	93	87-99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human IL-19 were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates* (n=5)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva* (n=5)	Human milk* (n=5)
1:2	Average % of Expected	102	100	100	98	103	99
	Range (%)	98-107	95-104	95-102	92-108	99-108	94-104
1:4	Average % of Expected	103	104	103	101	101	99
	Range (%)	97-110	99-108	98-108	95-112	95-109	95-102
1:8	Average % of Expected	101	105	105	102	99	97
	Range (%)	96-107	97-114	97-109	95-116	91-106	92-100
1:16	Average % of Expected	99	102	100	98	94	93
	Range (%)	94-101	98-106	94-107	92-110	90-103	86-97

*Samples were diluted prior to assay.

SENSITIVITY

Fifty-six assays were evaluated and the minimum detectable dose (MDD) of human IL-19 ranged from 2.2-12.2 pg/mL. The mean MDD was 5.4 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 human IL-19 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine/Human Milk - Samples from apparently healthy volunteers were evaluated for the presence of human IL-19 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=69)	58.7	4	ND-81.9
EDTA plasma (n=69)	56.9	4	ND-83.9
Heparin plasma (n=69)	65.8	6	ND-90.5

ND=Non-detectable

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Saliva (n=19)	935	84	ND-2954
Urine (n=18)	130	11	ND-195

ND=Non-detectable

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Human milk (n=5)	115	25.3-177	64.0

Cell Culture Supernates:
Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal bovine serum, 5 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 for the 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human IL-19.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	216	ND
Stimulated	235	347

ND-Non-detectable

Human monocytes were cultured in RPMI supplemented with 10% fetal bovine serum, 25 ng/mL recombinant human GM-CSF, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured stimulated with 50 ng/mL LPS for the final 24 hours. An aliquot of the cell culture supernate was removed, assayed for human IL-19, and measured 5084 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human IL-19.

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 IL-19 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

IL-10
IL-10 Rα
IL-10 Rβ
IL-20
IL-20 Rα
IL-20 Rβ
IL-22
IL-22 R
IL-24
IL-26

Recombinant mouse:

IL-10
IL-10 Rα
IL-10 Rβ
IL-19
IL-20
IL-20 Rα
IL-20 Rβ
IL-22 R
IL-24

Other recombinants:

canine IL-10
equine IL-10
feline IL-10
guinea pig IL-10
viral CMV IL-10
rat IL-10
rat IL-22

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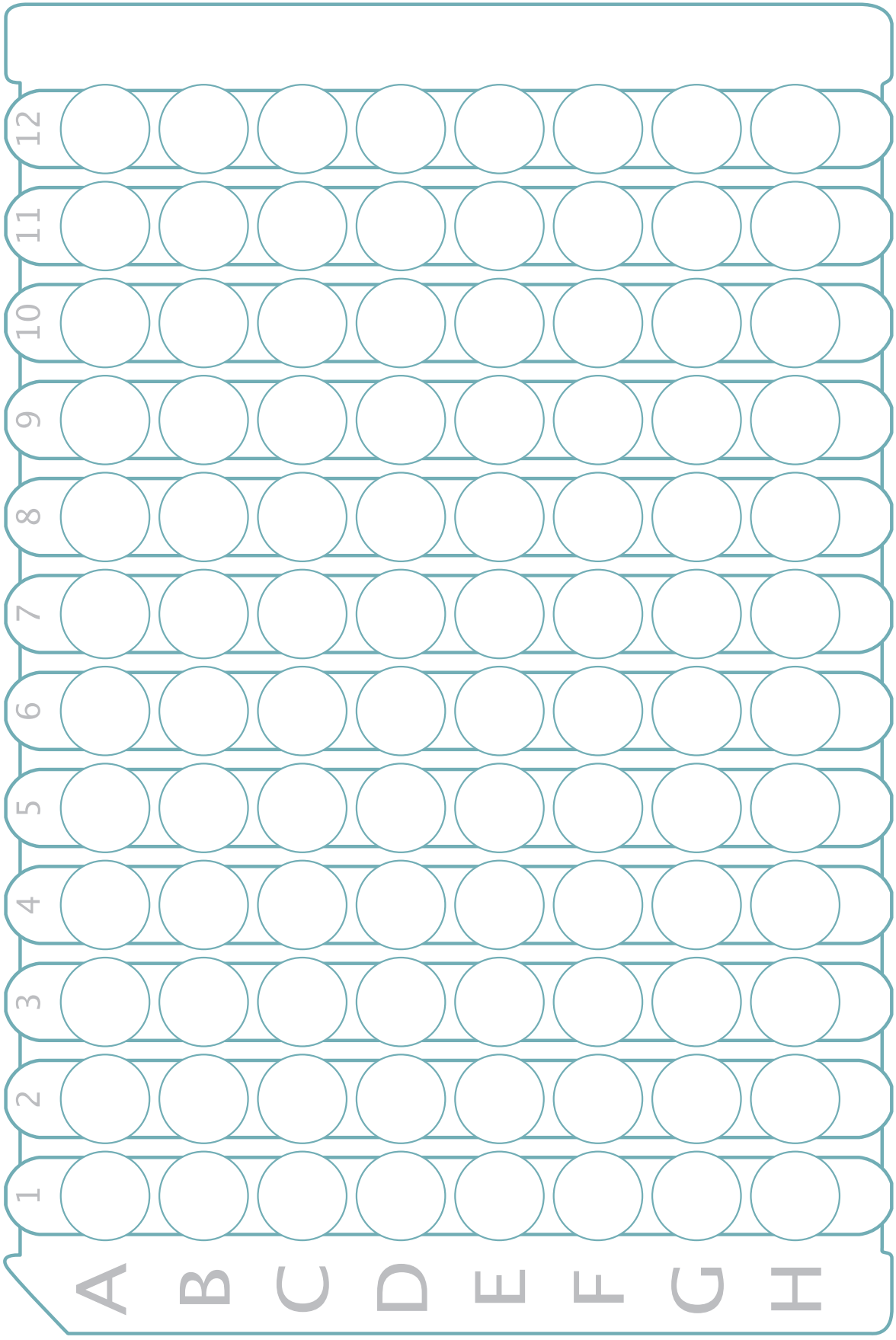
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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