Quantikine® ELISA

Human CCL3/MIP-1α Immunoassay

Catalog Number DMA00
SMA00
PDMA00

For the quantitative determination of human Macrophage Inflammatory Protein 1 alpha (MIP-1α) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

Macrophage Inflammatory Protein-1 alpha (MIP-1α) is a member of the b or CC subfamily of chemokines. Additional chemokine subfamilies, classified according to their cysteine motifs, include the α or CXC and the γ or C subfamilies. Chemokines share from 20 to greater than 90 percent amino acid (aa) sequence identity. Most chemokines have been shown to possess chemoattractant activity and to play key roles in immunoregulatory and inflammatory processes (1-3).

Human MIP-1α cDNA encodes a 92 aa residue precursor protein with a 22 aa residue signal peptide that is cleaved to generate the secreted mature protein. MIP-1α has been shown to be produced by activated T cells, B cells, monocytes, mast cells, neutrophils, Langerhans cells, astrocytes, endothelial cells, fibroblasts and smooth muscle cells (2-7). The gene for MIP-1α has been mapped to chromosome 17, along with all other human b chemokine genes (2).

Like other β chemokines, MIP-1α is a monocyte chemoattractant (1, 2). In addition, MIP-1α has also been reported to have differential chemoattractant and pro-adhesive effects on T-lymphocytes (8, 9), NK cells (10), cytotoxic T cells, B cells (11), basophils (12), and eosinophils (13). MIP-1α has been identified as a stem cell inhibitor (SCI) that can inhibit the proliferation of hematopoietic progenitor cells both in vitro and in vivo (14-16). MIP-1α, MIP-1β, and RANTES have been implicated as the major HIV-suppressive factors produced by $CD8^+$ T cells (17).

The Quantikine Human CCL3/MIP-1α Immunoassay is a 3.5-4.5 hour solid phase ELISA designed to measure MIP-1α levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human MIP-1α and antibodies raised against the recombinant protein. Results obtained for naturally occurring human MIP-1α 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 of natural human MIP-1α.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human MIP-1α has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any MIP-1α present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human MIP-1α 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 MIP-1α 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, further 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 #	CATALOG # DMA00	CATALOG # SMA00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human MIP-1α Microplate	890099	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human MIP-1α.	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 MIP-1α Standard	890101	1 vial	6 vials	Recombinant human MIP-1α in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human MIP-1α Conjugate	890100	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human MIP-1α conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1U	895138	1 vial	6 vials	6 mL/vial of a buffered protein base with preservatives. *Contains a precipitate. Mix well before and during use. For serum/plasma samples.*	
Calibrator Diluent RD5K	895119	2 vials	12 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6F	895018	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.

DMA00 contains sufficient materials to run an ELISA on one 96 well plate.
SMA00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDMA00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please 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 and samples.
- Human MIP-1α Controls (optional; R&D Systems, Catalog # QC20).

PRECAUTIONS

Calibrator Diluent RD6F 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. 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, heparin, or citrate 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.

SAMPLE PREPARATION

Cell culture supernate samples may require up to a 50-fold dilution prior to assay. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5K.

REAGENT PREPARATION

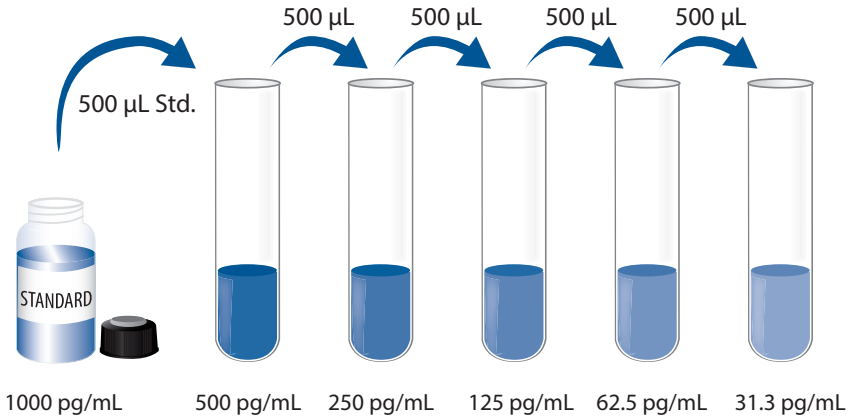
Bring all reagents to room temperature before use.

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.

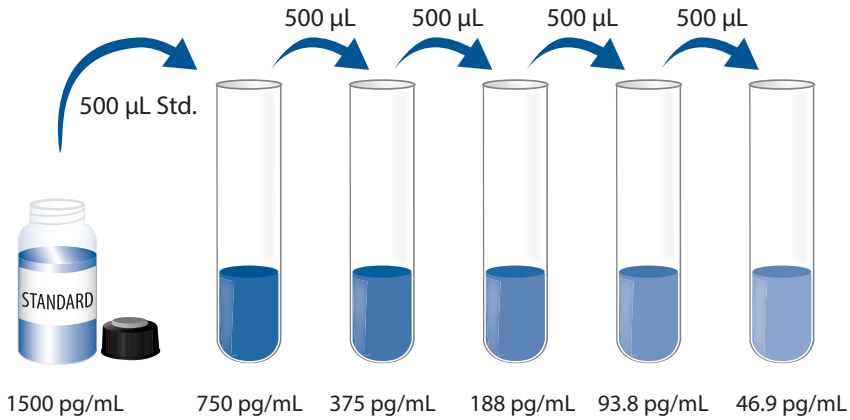
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human MIP-1α Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MIP-1α Standard with Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6F (*for serum/plasma samples*). This reconstitution produces a stock solution of 1000 pg/mL or 1500 pg/mL, respectively. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

For Cell Culture Supernate Samples: Pipette 500 µL of Calibrator Diluent RD5K into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human MIP-1α Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5K serves as the zero standard (0 pg/mL).



For Serum/Plasma Samples: Pipette 500 µL of Calibrator Diluent RD6F into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human MIP-1α Standard (1500 pg/mL) serves as the high standard. Calibrator Diluent RD6F 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. **For Serum/Plasma Samples Only:** Add 50 µL of Assay Diluent RD1U to each well. *Assay Diluent RD1U will have a precipitate present. Mix well before and during use.*
4. Add 200 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 twice for a total of three 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 MIP-1α Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1 hour at room temperature.
   For Serum/Plasma Samples: 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 20 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.).

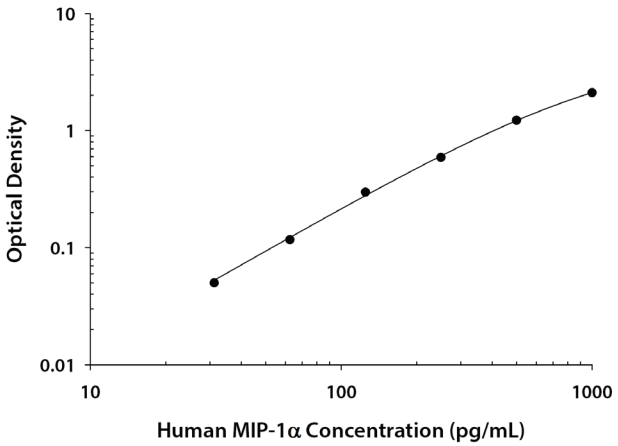
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human MIP-1α concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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

TYPICAL DATA

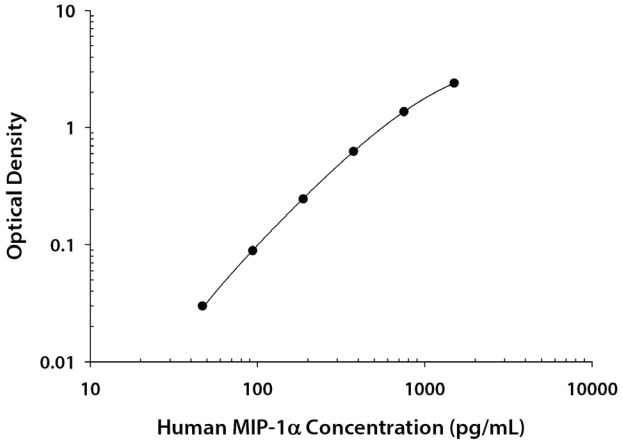
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.022 0.026	0.024	—
31.3	0.072 0.076	0.074	0.050
62.5	0.136 0.146	0.141	0.117
125	0.323 0.322	0.322	0.298
250	0.590 0.637	0.614	0.590
500	1.254 1.237	1.246	1.222
1000	2.147 2.112	2.130	2.106

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.032	0.030	—
46.9	0.061 0.058	0.060	0.030
93.8	0.120 0.118	0.119	0.089
187.5	0.291 0.260	0.276	0.246
375	0.647 0.664	0.656	0.626
750	1.386 1.402	1.394	1.364
1500	2.359 2.498	2.428	2.398

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)	116	442	726	53.6	313	623
Standard deviation	3.30	5.90	14.0	3.70	13.4	25.7
CV (%)	2.8	1.3	1.9	6.9	4.3	4.1

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)	140	688	1084	50.7	306	613
Standard deviation	12.5	60.4	55.4	5.90	15.1	37.8
CV (%)	8.9	8.8	5.1	11.6	4.9	6.2

RECOVERY

The recovery of human MIP-1α spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media	100	88-117%
Serum	95	87-113%
EDTA plasma	96	81-109%
Heparin plasma	98	82-121%
Citrate plasma	94	85-104%

SENSITIVITY

The minimum detectable dose (MDD) of human MIP-1α is typically less than 10 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.

LINEARITY

To assess linearity of the assay, samples were spiked with high concentrations of human MIP-1α and diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=8)	Serum (n=3)	EDTA plasma (n=3)	Heparin plasma (n=3)	Citrate plasma (n=3)
1:2	Average % of Expected	106	105	106	101	106
	Range (%)	102-108	99-109	103-110	92-109	105-106
1:4	Average % of Expected	109	111	112	111	106
	Range (%)	105-115	104-118	108-117	96-121	105-108
1:8	Average % of Expected	105	111	112	118	108
	Range (%)	100-110	96-118	106-124	112-122	101-122
1:16	Average % of Expected	104	116	103	115	111
	Range (%)	96-112	101-127	94-109	109-125	98-124

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant human MIP-1a produced at R&D Systems.

SAMPLE VALUES

Serum - Forty samples from apparently healthy volunteers were evaluated for the presence of human MIP-1α in this assay. All samples measured less than the lowest standard, 46.9 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated and stimulated with 10 µg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 5 and assayed for levels of natural human MIP-1α.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	27,440	2370
Stimulated	407	175

SPECIFICITY

This assay recognizes natural and recombinant human MIP-1α.

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 MIP-1α control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

EGF
ENA-78
G-CSF
GM-CSF
GROα
I-309
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-6
IL-7
IL-8
IP-10
LIF
MCP-1
MIP-1β
RANTES
SCF
TGF-α
TGF-β1
TNF-α
TNF-β

Recombinant mouse:

C10
EGF
GM-CSF
IL-1β
IL-3
IL-4
IL-5
IL-6
IL-7
JE/MCP-1
MIP-1α
MIP-1β

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
human TGF-β1
porcine TGF-β1
porcine TGF-β1.2
porcine TGF-β2
porcine PDGF

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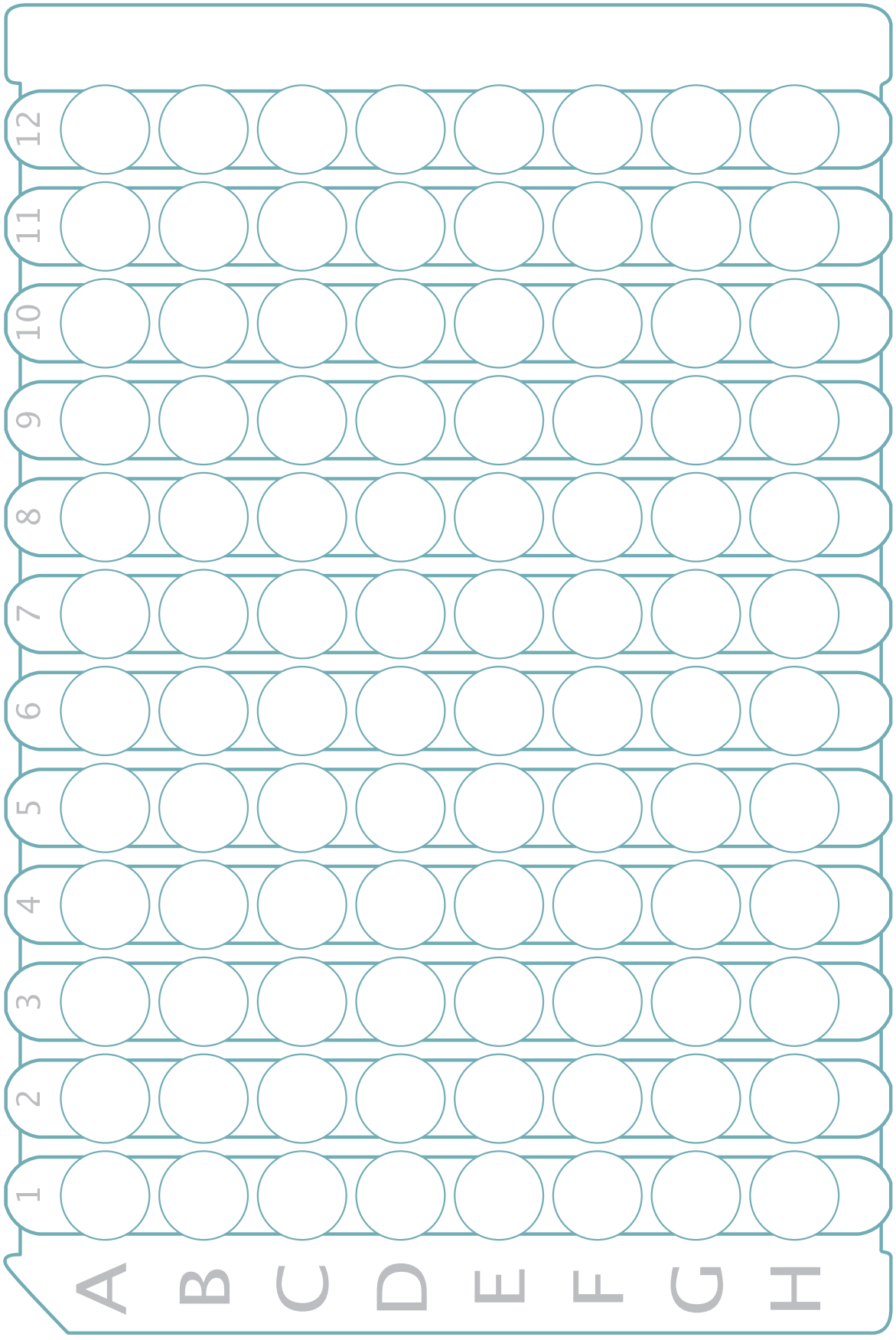
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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