bio-techne®

R&D SYSTEMS

Saliva Sensitive

Quantikine™ QuicKit™ ELISA

Human CCL19/MIP-3β Immunoassay

Catalog Number QK0361
SK0361
PK0361

For the quantitative determination of human MIP-3β 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

Macrophage Inflammatory Protein-3 beta (MIP-3β), also known as CCL19, is a chemokine that plays a pivotal role in the immune system by mediating the migration and positioning of immune cells. It primarily acts on dendritic cells and T cells, guiding their movement towards lymphoid tissues, which is crucial for initiating and sustaining immune responses (1). MIP-3β binds to the CCR7 receptor, a critical interaction that regulates the homing of T cells and dendritic cells to lymph nodes (2).

In the context of immuno-oncology, MIP-3β plays a crucial role in tumor immunology. The tumor microenvironment often manipulates chemokine signals to evade immune detection. By understanding and potentially modulating MIP-3β levels, researchers aim to enhance the recruitment of immune cells to tumors, thereby boosting anti-tumor immunity (3). Increased MIP-3β expression has been associated with better prognosis in certain cancers due to its role in attracting effector immune cells to the tumor site (4).

MIP-3β also holds significance in broader immunology. Its role in immune cell trafficking is essential for mounting effective immune responses against pathogens and for the maintenance of immune surveillance. Alterations in MIP-3β expression or function can lead to immune dysregulation and are implicated in various immunological disorders (5).

Utilizing ELISA to measure MIP-3β can offer valuable insights into immune cell dynamics and the effectiveness of immunotherapies. For example, monitoring MIP-3β levels could help evaluate the immune cell infiltration in tumors and the impact of immune-modulating treatments (6).

The Quantikine™ QuicKit™ Human CCL19/MIP-3β Immunoassay is a one-step, 80-minute solid phase ELISA designed to measure human MIP-3β levels in cell culture supernates, serum, and plasma. It contains recombinant human MIP-3β and antibodies raised against the recombinant protein. Results obtained for naturally occurring human MIP-3β showed linear curves that were parallel to the standard curves obtained using the recombinant QuicKit standards. These results indicate that this kit can be used to determine relative mass values for natural MIP-3β .

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled polyclonal capture antibody and an enzyme-linked polyclonal detection antibody, specific for human MIP-3β. Following a wash to remove any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of MIP-3β bound. 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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the 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™ QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step 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 # QK0361	CATALOG # SK0361	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
QuicKit™ Coated Microplate	899063	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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 CCL19/MIP-3β Standard	899569	2 vials	12 vials	Recombinant human MIP-3β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for the reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human CCL19/MIP-3β Capture Ab Concentrate	899567	1 vial	6 vials	Lyophilized tagged polyclonal antibody specific for human MIP-3β.	May be stored for up to 1 month at 2-8 °C.*
Human CCL19/MIP-3β Detection Ab Concentrate	899568	1 vial	6 vials	400 µL of a polyclonal antibody specific for human MIP-3β conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1-21	895215	1 vial	6 vials	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-20	895346	1 vial	6 vials	21 mL of a buffered protein base with preservatives. *Use diluted 1:2 in this assay.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
TMB ELISA Substrate	642736	1 vial	6 vials	12 mL of a TMB ELISA substrate.	
ELISA Stop Solution	642827	1 vial	6 vials	12 mL of an acid solution.	
Plate Sealers	N/A	4 strips	8 strips	Adhesive strips.	

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

QK0361 contains sufficient materials to run an ELISA on one 96 well plate.
SK0361(SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, # PK0361). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak has enough reagents to assay 50 microplates (96 wells/plate). Although the package inserts are the same as those for the single kit inserts, there are minor differences related to the number of reagents and their container sizes.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  Note: *Additional wash buffer is available for purchase (R&D Systems®, # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
QuicKit™ Coated Microplate	899063	50 plates
Human CCL19/MIP-3β Standard*	899569	25 vials
Human CCL19/MIP-3β Capture Ab Concentrate	899567	50 vials
Human CCL19/MIP-3β Detection Ab Concentrate	899568	50 vials
Assay Diluent RD1-21	895215	50 vials
Calibrator Diluent RD5-20	895346	50 vials
Wash Buffer Concentrate	895126	9 bottles
TMB ELISA Substrate	642736	50 vials
ELISA Stop Solution	642827	50 vials
Plate Sealers	N/A	100 sheets

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

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
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm
- **Polypropylene** test tubes for dilution of standards
- Human MIP-3β Controls (optional; Catalog # QC318)

PRECAUTIONS

MIP-3β 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.

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.*
Grossly icteric samples are not suitable for use in this assay.

SAMPLE PREPARATION

Cell culture supernates samples require a 2-fold dilution. A suggested 2-fold dilution is 75 µL of sample + 75 µL of Calibrator Diluent RD5-20 (diluted 1:2)*.

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 50 µL of sample + 150 µL of Calibrator Diluent RD5-20 (diluted 1:2)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *MIP-3β is found in saliva. Wear a face mask and gloves be used to protect kit reagents from contamination.*

Human MIP-3β Capture Ab Concentrate - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MIP-3β Capture Ab Concentrate with Assay Diluent RD1-21. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

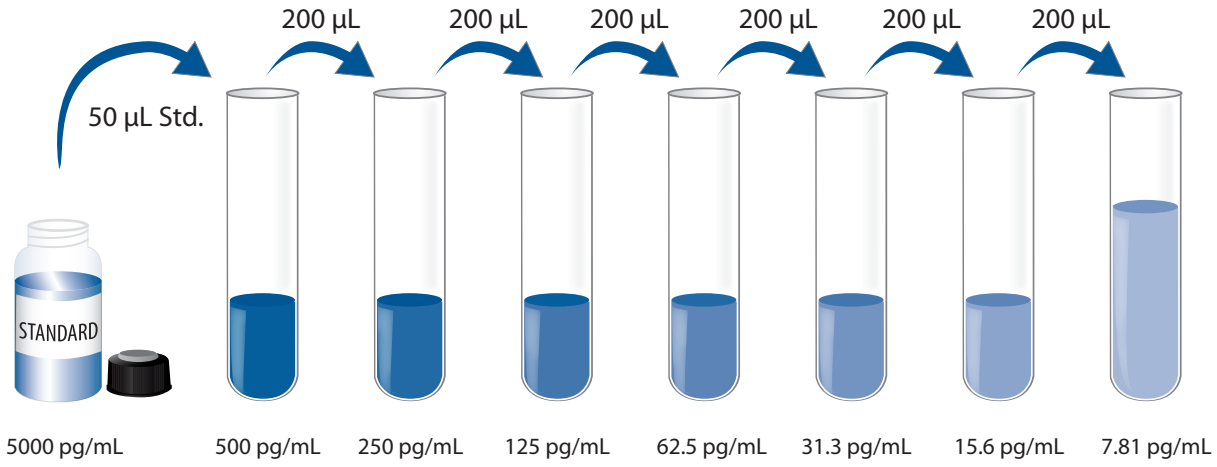
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1-21. For a full plate, add 300 µL of reconstituted Human MIP-3β Capture Ab Concentrate and 300 µL of Human MIP-3β Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1-21.

Calibrator Diluent RD5-20 (diluted 1:2) - Add 20 mL of Calibrator Diluent RD5-20 to 20 mL of deionized or distilled water to prepare 40 mL of Calibrator Diluent RD5-20 (diluted 1:2).

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.

Human MIP-3β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human MIP-3β Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.
Note: *Do not use rocker or vortex for mixing.*

Use polypropylene tubes. Pipette 450 µL of Calibrator Diluent RD5-20 (diluted 1:2) into the 500 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-20 (diluted 1:2) 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: *MIP-3β is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 50 µL of standard, control, or sample* per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm.
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 100 µL of TMB Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 100 µL of ELISA 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.
8. 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

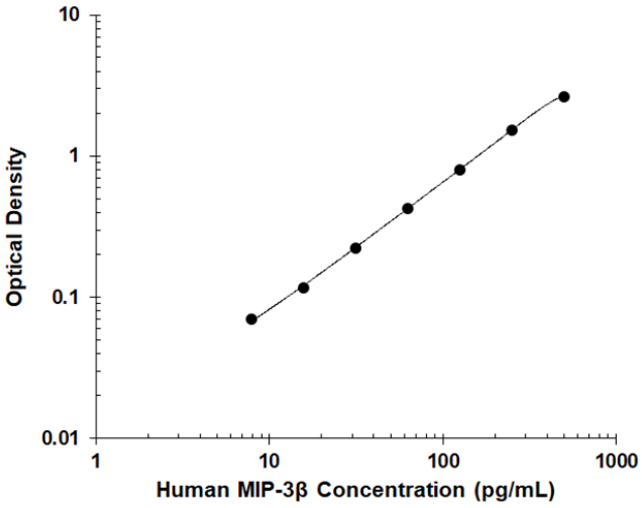
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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-3β 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

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	0.022 0.022	0.022	—
7.81	0.067 0.073	0.070	0.048
15.6	0.115 0.119	0.117	0.095
31.3	0.218 0.227	0.223	0.201
62.5	0.419 0.432	0.426	0.404
125	0.799 0.805	0.802	0.780
250	1.513 1.550	1.532	1.510
500	2.636 2.709	2.673	2.651

PRECISION

Intra-Assay Precision (Precision within an assay)
Two samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least five technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	42.4	326	45.8	338
Standard deviation	3.15	18.8	3.36	26.7
CV (%)	7.4	5.8	7.3	7.9

RECOVERY

The recovery of human MIP-3β spiked to three different levels in samples throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	112	106-117%
Serum (n=2)	98	94-103%
EDTA plasma (n=2)	99	91-107%
Heparin plasma (n=2)	98	93-102%

SENSITIVITY

Fifteen assays were evaluated and the minimum detectable dose (MDD) of human MIP-3β ranged from 0.155-2.56 pg/mL. The mean MDD was 0.695 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human MIP-3β were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n= 2)	EDTA plasma (n= 2)	Heparin plasma (n= 2)
1:2	Average % of Expected	93	102	103	104
	Range (%)	89-96	102-103	102-104	103-105
1:4	Average % of Expected	91	102	104	103
	Range (%)	87-95	100-105	104-104	101-105
1:8	Average % of Expected	90	102	101	102
	Range (%)	86-94	98-106	98-104	101-102
1:16	Average % of Expected	88	99	100	97
	Range (%)	85-91	97-102	96-103	96-98

CALIBRATION

This immunoassay is calibrated against a highly purified recombinant human MIP-3β produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma- Samples from apparently healthy volunteers were evaluated for the presence of human MIP-3β in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=10)	108	56.0-172	37.9
EDTA plasma (n=10)	112	65.3-179	38.0
Heparin plasma (n=10)	120	76.6-184	39.5

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMCs) (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. PBMCs were treated with 10 µg/mL PHA for 48 hours prior to conditioned media harvest. Aliquots of the culture supernates were removed and assayed for levels of human MIP-3β.

Donor	(pg/mL)
Donor 1	212
Donor 2	103

SPECIFICITY

This assay recognizes natural and recombinant human MIP-3β.

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 low level recombinant human MIP-3β control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

6Ckine
Eotaxin-3
GROβ
MIP-1β
MIP-3α
RANTES

Other recombinants:

mouse MIP-3β
rat CCR7

Recombinant rat CCL19 cross-reacts approximately < 1% in this assay.

REFERENCES

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4. Chang J.E. *et al.* (2015) Trends Immunol. **36**:30.
5. Vissers J.L. *et al.* (2001) J Leukoc Biol. **69**:785.
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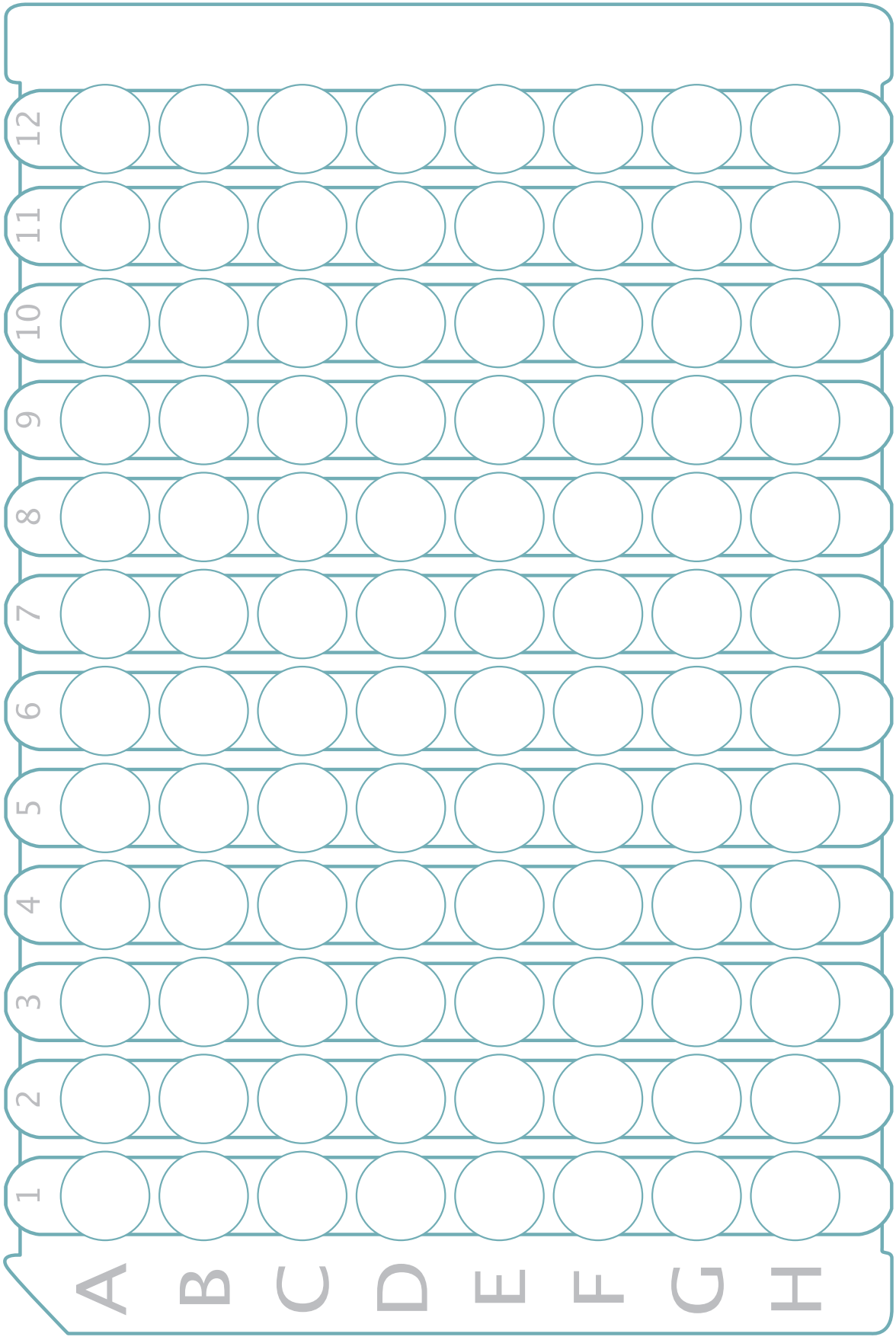
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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