Quantikine® ELISA

Mouse IL-10 Immunoassay

Catalog Number M1000B
SM1000B
PM1000B

For the quantitative determination of mouse Interleukin 10 (IL-10) 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-10 (IL-10), also known as cytokine synthesis inhibitory factor (CSIF), is the charter member of the IL-10 α-helical cytokine family that also includes IL-19, IL-20, IL-22, IL-24, and IL-26/AK155 (1-3). IL-10 is secreted by many activated hematopoietic cell types as well as hepatic stellate cells, keratinocytes, and placental cytotrophoblasts. Whereas human IL-10 is active on mouse cells, mouse IL-10 does not act on human cells (4, 5). Mature mouse IL-10 shares 85% amino acid sequence identity with rat IL-10 and 70-77% with bovine, canine, equine, feline, human, ovine, and porcine IL-10. It contains two intrachain disulfide bridges and is expressed as a 36 kDa noncovalently-associated homodimer (4, 6, 7).

IL-10 mediates its biological activities through a heteromeric receptor complex composed of the type II cytokine receptor subunits IL-10 Rα and IL-10 Rβ. IL-10 Rα is a 110 kDa transmembrane glycoprotein that is expressed on lymphocytes, NK cells, macrophages, monocytes, astrocytes, intestinal epithelial cells, cytotrophoblasts, and activated hepatic stellate cells (8-13), while the 75 kDa transmembrane IL-10 Rβ is widely expressed (14, 15). The IL-10 dimer binds to two IL 10 Rα chains, triggering recruitment of two IL-10 Rβ chains (14, 15). IL-10 Rβ does not bind IL-10 directly but is required for signal transduction. IL-10 Rβ also associates with IL-20 Rα, IL-22 Rα1, or IL-28 Rα to form the receptor complexes for IL-22, IL-26, IL-28, and IL-29 (16-18).

The involvement of IL-10 in immunoregulation includes both suppressive and stimulatory effects. It functions as an anti-inflammatory cytokine by inhibiting the expansion and activation of Th1 cells and Th17 cells (19-21) and by promoting the development of M2 macrophages (21). Its expression by immunosuppressive regulatory T cells (Treg) and regulatory B cells is important for Treg proliferation (19). Within a tumor microenvironment, however, IL-10 inhibits the expansion of Treg as well as myeloid-derived suppressor cells (22, 23). IL-10 induces the intratumoral accumulation and activation of $CD8^+$ T cells (24, 25). IL-10 exerts protective effects including limiting tissue damage in arthritic inflammation (19) and promoting muscle regeneration after injury (21), but it also contributes to the persistence of viral infections (26). The levels of IL-10 are elevated in Sjogren's syndrome (saliva), primary CNS lymphoma (cerebrospinal fluid), and ovarian cancer (serum and ascites) (27-29). Its levels are decreased in the serum in patients with recurrent heart attacks or during preeclampsia and also in the seminal fluid of infertile men (30-32).

The Quantikine® Mouse IL-10 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-10 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-10 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant mouse IL-10 accurately. Results obtained using natural mouse IL-10 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 mouse IL-10.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monolonal antibody specific for mouse IL-10 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-10 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-10 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells. The enzyme reaction yields a blue product that turns yellow when the Stop Solution is added. The intensity of the color measured is in proportion to the amount of IL-10 bound in the initial step. The sample values are then read off the standard curve.

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, further dilute the samples with 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.
- 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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # M1000B	CATALOG # SM1000B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-10 Microplate	894508	2 plates	6 plates	96 well polystyrene microplates (12 strips of 8 wells) coated with monoclonal antibody specific for mouse IL-10.	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.*
Mouse IL-10 Standard	894510	3 vials	9 vials	Recombinant mouse IL-10 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard and control for each assay. Discard after use.
Mouse IL-10 Control	894511	3 vials	9 vials	Recombinant mouse IL-10 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Mouse IL-10 Conjugate	894509	1 vial	3 vials	23 mL/vial of a polyclonal antibody specific for mouse IL-10 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895038	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5T	895175	2 vials	6 vials	21 mL/vial of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials	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	3 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	3 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895174	1 vial	3 vials	23 mL/vial of diluted hydrochloric acid.	
Plate Sealers	N/A	8 strips	24 strips	Adhesive strips.	

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

M1000B contains sufficient materials to run ELISAs on two 96 well plates.
SM1000B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM1000B). 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.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.

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 - Allow blood samples to clot for 2 hours at room temperature before centrifuging for 20 minutes at 2000 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 20 minutes at 2000 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.*

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution into Calibrator Diluent RD5T. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5T.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

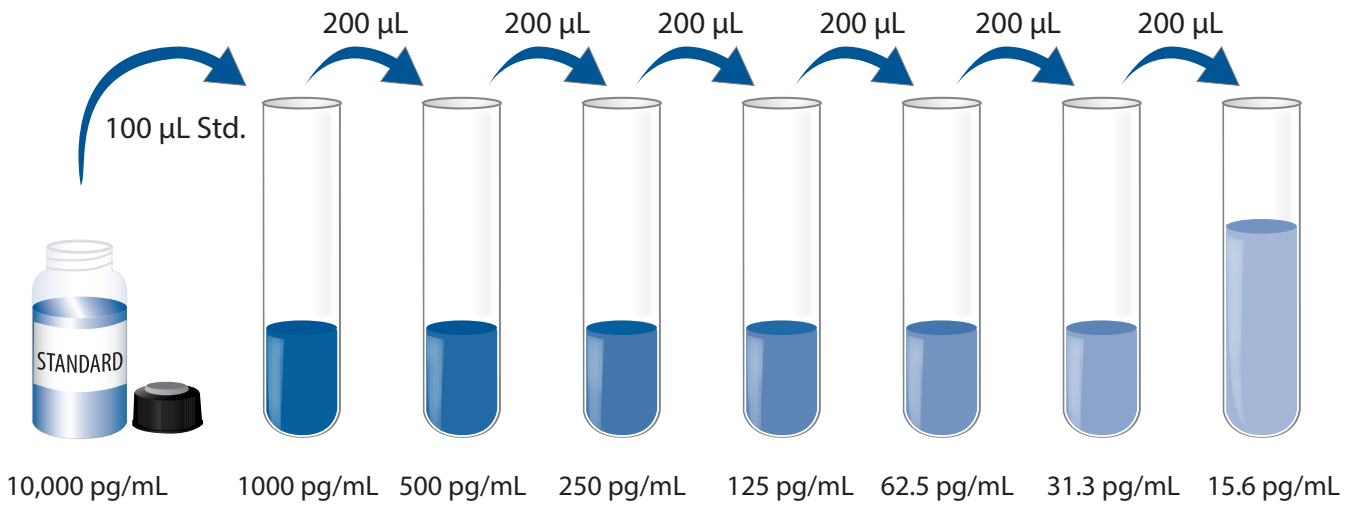
Mouse IL-10 Kit Control - Reconstitute the control with 1.0 mL deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. To prepare enough Wash Buffer for one plate, add 20 mL 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. 100 µL of the resultant mixture is required per well.

Mouse IL-10 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-10 Standard with deionized or distilled water. Do not substitute other diluents. This reconstitution produces a stock solution of 10,000 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5T into the 1000 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 1000 pg/mL standard serves as the high standard. Calibrator Diluent RD5T 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 samples, standards, and control be assayed in duplicate.

1. Prepare reagents, samples, standards, and control as directed by 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 Assay Diluent RD1W 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm ± 50 rpm. 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 100 µL of Mouse IL-10 Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 100 µL of Stop Solution to each well. 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

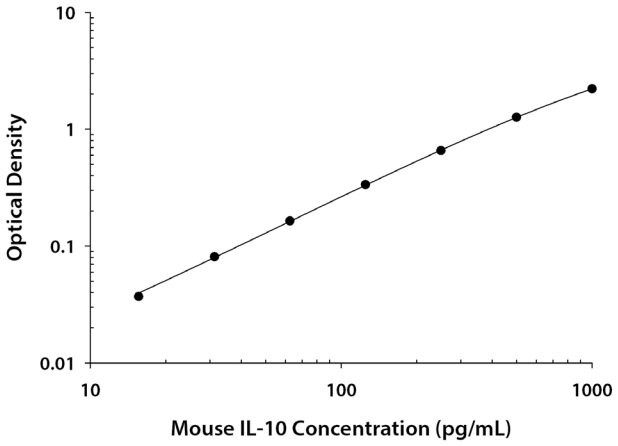
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 mouse IL-10 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	0.049 0.050	0.050	—
15.6	0.083 0.091	0.087	0.037
31.3	0.127 0.134	0.131	0.081
62.5	0.209 0.219	0.214	0.164
125	0.386 0.386	0.386	0.336
250	0.697 0.715	0.706	0.656
500	1.312 1.318	1.315	1.265
1000	2.255 2.266	2.261	2.211

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 over two lots of kit components. Assays were performed by at least three technicians.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	76.3	123	815	71.4	115	809
Standard deviation	2.56	2.85	17.0	5.39	7.64	55.5
CV (%)	3.4	2.3	2.1	7.5	6.6	6.9

RECOVERY

The recovery of mouse IL-10 spiked to three levels throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture samples (n=4)	99	95-103%
Serum* (n=4)	89	85-96%
EDTA plasma* (n=4)	91	86-99%
Heparin plasma* (n=4)	89	82-98%

*Samples were diluted prior to assay as directed in the Sample Preparation section.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse IL-10 in each matrix were diluted with calibrator diluent and then assayed.

		Cell culture samples (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	102	106	107	106
	Range (%)	98-105	102-114	104-111	104-107
1:4	Average % of Expected	100	109	110	115
	Range (%)	95-106	102-118	102-115	113-117
1:8	Average % of Expected	100	111	110	116
	Range (%)	92-107	102-120	104-117	113-118
1:16	Average % of Expected	99	113	113	116
	Range (%)	89-111	104-120	108-118	110-119

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Fifty-four assays were evaluated and the minimum detectable dose (MDD) of mouse IL-10 ranged from 0.625-5.22 pg/mL. The mean MDD was 1.97 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 mouse IL-10 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Twenty samples were evaluated for the presence of mouse IL-10 in this assay. No detectable levels were observed.

Cell Culture Supernates:
Mouse spleens were removed, rinsed in 1X PBS, and kept on ice in PBS. Organs were then homogenized using a tissue homogenizer. Cells were seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 1 µg/mL LPS for 3 days. Aliquots of the cell culture supernates were removed and assayed for levels of mouse IL-10.

Tissue Type	Unstimulated (pg/mL)	Stimulated (pg/mL)
Spleen	ND	77.1

ND=Non-detectable

Tissues from individual mice were removed and rinsed in PBS and kept on ice. The tissue was homogenized using a tissue homogenizer and seeded into media containing RPMI 1640 supplemented with 10% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were stimulated with 100 ng/mL recombinant mouse IFN-γ and 1 µg/mL LPS for 4 days. An aliquot of the cell culture supernate was removed, assayed for mouse IL-10, and measured 515 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-10.

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 mouse IL-10 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

IFN-α/β R2
IFN-γ R1
IFN-γ R2
IL-10 R
IL-10 Rα
IL-10 Rβ
IL-19
IL-20
IL-22
IL-22 R
IL-24

Other recombinants:

canine IL-10
equine IL-10
feline IL-10
human IL-10
porcine IL-10
viral IL-10

Recombinant rat IL-10 cross-reacts approximately 0.8% in this assay.

Recombinant cotton rat IL-10 cross-reacts approximately 0.1% in this assay.

Recombinant guinea pig IL-10 cross-reacts approximately 16% in this assay.

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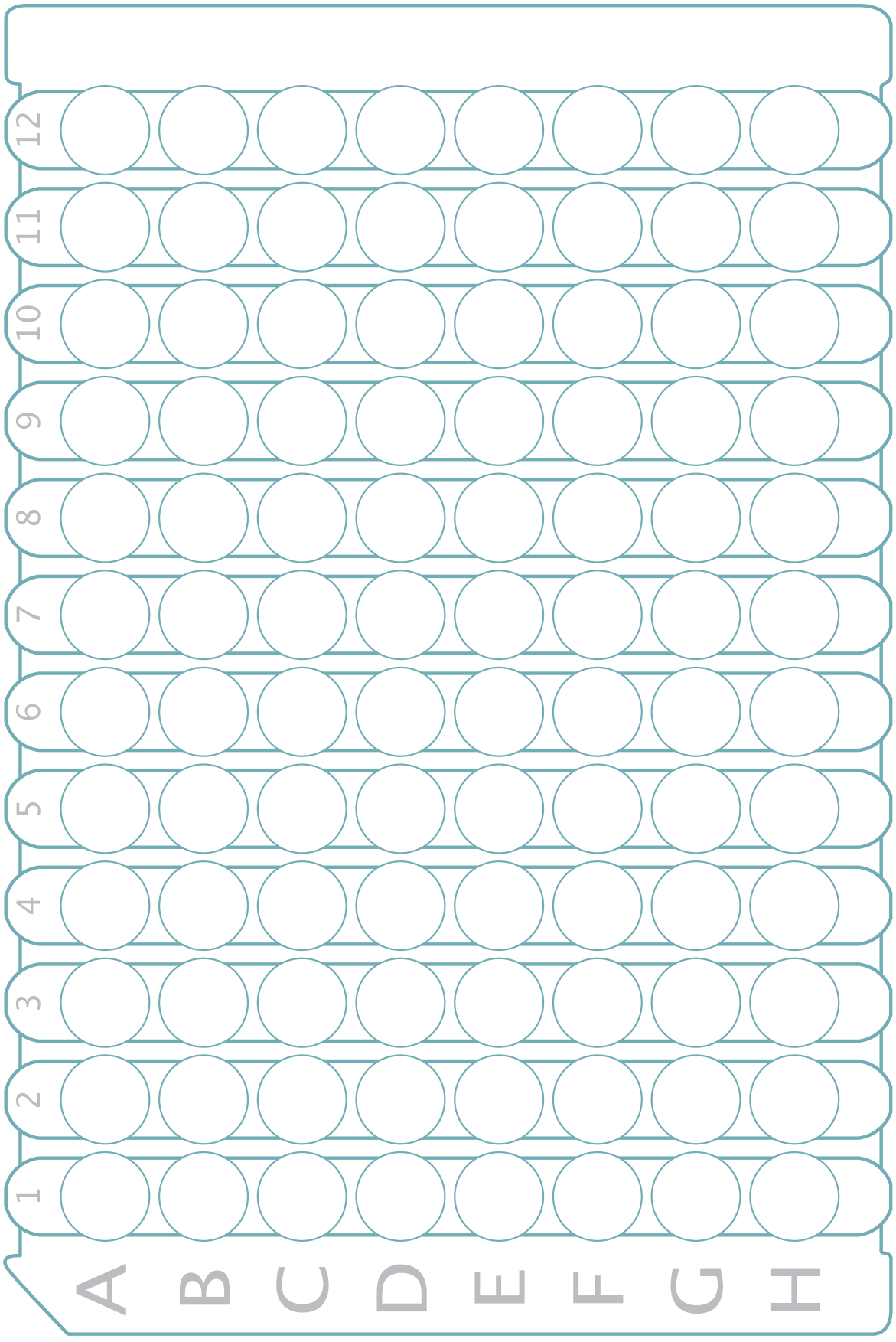
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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