Quantikine™ ELISA

Mouse IL-17 Immunoassay

Catalog Number M1700-1
SM1700
PM1700

For the quantitative determination of mouse Interleukin 17 (IL-17) 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.

TABLE OF CONTENTS

SECTION PAGE

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INTRODUCTION

Mouse Interleukin 17 (IL-17; also known as IL-17A and CTLA-8) is a 21 kDa, variably glycosylated polypeptide that belongs to the IL-17 family of cytokines containing a cysteine-knot fold (1-3). Its sequence was originally isolated from an activated hybridoma created from the fusion of a mouse cytotoxic and rat T cell lymphoma cell line (2-5). It is synthesized as a 158 amino acid (aa) precursor that contains a 25 aa signal sequence and a 15 kDa, 133 aa mature segment (5). In both mouse and human, there is one conserved N-linked glycosylation site that likely contributes 5 kDa to its native molecular weight. IL-17A forms both a 35-38 kDa homodimer, and a 45-48 kDa heterodimer with IL-17F (6, 7). Mature mouse IL-17A is 61% and 89% aa identical to human and rat IL-17A, respectively (4, 5, 8). While rodent and human mature sequences show modest aa sequence identity, human IL-17 is active on both mouse and rat cells (5, 9). Cells known to produce IL-17 are the $CD4^+$ Th17 T cells, Paneth cells, $GR1^+CD11b^+$ myeloid suppressor cells, CD27-γδ T cells, $CD1^+NK1.1^-$ iNKT cells and $CD3^-CD4^+$ LTi-like cells (3, 5, 6, 10-12).

A high affinity receptor for mouse IL-17 has been reported, and appears to be a heteromultimer of IL-17RA and IL-17RC, likely in a 2:1 ratio (1). IL-17RA is a 130 kDa, type I transmembrane glycoprotein that bears no resemblance to members of the cytokine, TNF or immunoglobulin receptor superfamily (2, 10, 13). IL-17RC is also a type I transmembrane protein, approximately 90-95 kDa in size, that shares less than 30% aa identity with IL-17RA (14, 15). Both receptors are needed for IL-17A and IL-17A/F activity. The two receptors appear to form a functional association following ligand binding to IL-17RA (1, 16).

IL-17 is best known for its participation in the recruitment and survival of neutrophils (3, 10, 17, 18). Its induction was initially described to be the result of antigen stimulation of dentritic cells, resulting in IL-23 secretion. In a TCR-independent event, IL-23 induces T cell production of IL-17 (3). Once secreted, IL-17 in the bone marrow would seem to induce stromal/fibroblast expression of both G-CSF and SCF (membrane form), an effect that increases neutrophil differentiation and activation. IL-17 may complement this by directly blocking neutrophil apoptosis, promoting greater circulating neutrophil numbers (17). In the tissues, IL-17 seems to promote neutrophil extravasation, principally through its effects on macrophages and endothelial cells (EC). On macrophages, IL-17 induces TNF-α, IL-1β and IL-6 production (19). TNF-α and IL-1β then act on local ECs to induce G-CSF secretion, an effect that is potentiated by IL-17 (20). IL-17 further contributes to neutrophil influx by inducing EC CXC chemokine release and NO production, which may increase vascular permeability (3, 9). IL-17 effects are not limited to neutrophils. In synovial joints, IL-17 upregulates RANKL expression on osteoblasts. This provides a stimulus for osteoclast formation and subsequent bone resorption (18).

The Quantikine™ Mouse IL-17 Immunoassay is a 4.5 hour solid phase ELISA designed to measure mouse IL-17 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant mouse IL-17 and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant mouse IL-17 accurately. Results obtained using natural mouse IL-17 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 mouse IL-17.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for mouse IL-17 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any IL-17 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for mouse IL-17 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-17 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, dilute the samples with calibrator diluent and repeat the assay.
- Any variation in 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.
- For best results, pipette reagents and samples into the center of each well.
- It is recommended that the samples be pipetted within 15 minutes.
- 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 # M1700-1	CATALOG # SM1700	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Mouse IL-17 Microplates	890669	1 plate	6 plates	96 well microplates (12 strips of 8 wells) coated with a monoclonal antibody specific for mouse IL-17.	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.*
Mouse IL-17 Standard	890670	1 vial	3 vials	Recombinant mouse IL-17 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.
Mouse IL-17 Control	890672	1 vial	3 vials	Recombinant mouse IL-17 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-17 Conjugate	899291	1 vial	6 vials	12.5 mL/vial of a polyclonal antibody specific for mouse IL-17 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-38	895301	1 vial	3 vials	12 mL/vial of a buffered protein solution with preservatives.	
Calibrator Diluent RD5T	895175	1 vial	3 vials	21 mL/vial of a buffered protein solution with preservatives.	
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	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	4 strips	24 strips	Adhesive strips.	

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

M1700-1 contains sufficient materials to run ELISAs on one 96 well plate.
SM1700 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PM1700). Refer to the PharmPak Contents section for specific vial counts.

PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- 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®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

PART	PART #	QUANTITY
Mouse IL-17 Microplates	890669	50 plates
Mouse IL-17 Standard*	890670	25 vials
Mouse IL-17 Control	890672	25 vials
Mouse IL-17 Conjugate	899291	50 vials
Assay Diluent RD1-38	895301	25 vials
Calibrator Diluent RD5T	895175	25 vials
Wash Buffer Concentrate	895126	9 bottles
Color Reagent A	895000	25 vials
Color Reagent B	895001	25 vials
Stop Solution	895174	25 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
- **Polypropylene** test tubes for dilution of standards

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 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 - 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.*
Grossly hemolyzed or lipemic samples are not suitable for use in this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

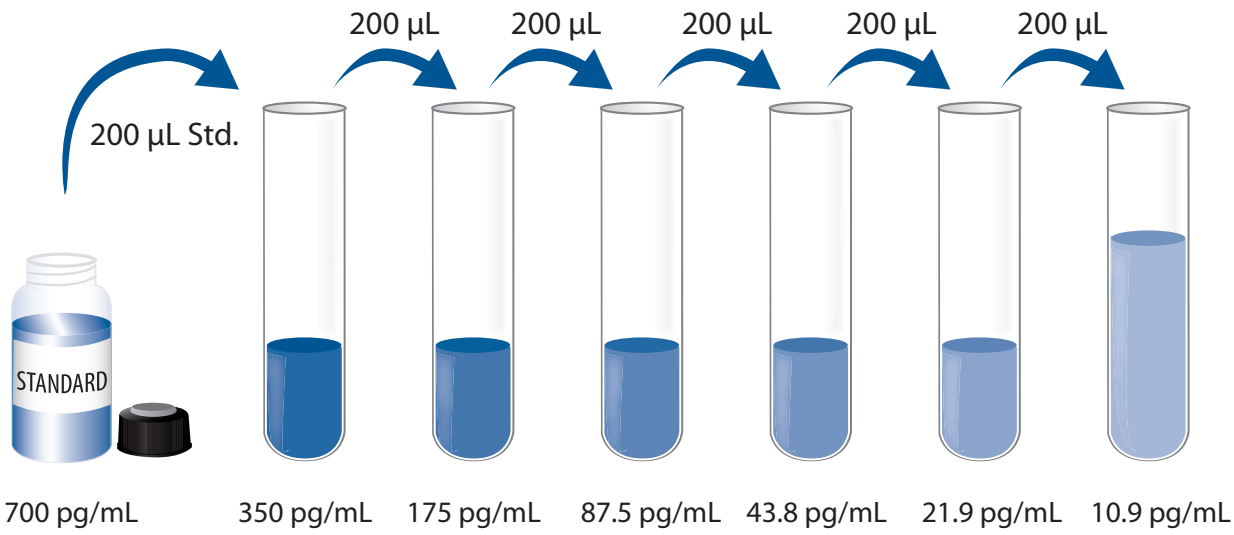
Mouse IL-17 Control - Reconstitute the control with 1 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate into 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. 100 µL of the resultant mixture is required per well.

Mouse IL-17 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Mouse IL-17 Standard with Calibrator Diluent RD5T. Do not substitute other diluents. This reconstitution produces a stock solution of 700 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5T into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Mouse IL-17 Standard (700 pg/mL) 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 standards, control, and samples be assayed in duplicate.

1. Prepare all reagents, standards, controls, 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 50 µL of Assay Diluent RD1-38 to each well.
4. Add 50 µL of standard, control, or sample per well. Mix by gently tapping the plate frame for 1 minute. 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 four times for a total of five 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 by inverting the plate and blotting it against clean paper towels.
6. Add 100 µL of Mouse IL-17 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 100 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature. **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.

CALCULATION OF RESULTS

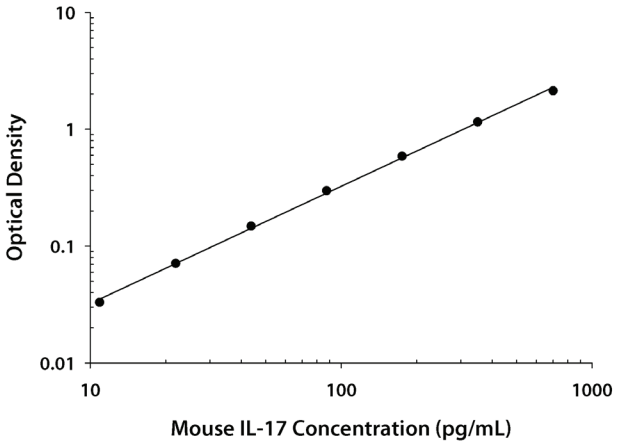
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 the graph. The data may be linearized by plotting the log of the mouse IL-17 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	0.032 0.032	0.032	—
10.9	0.064 0.066	0.065	0.033
21.9	0.103 0.103	0.103	0.071
43.8	0.177 0.183	0.180	0.148
87.5	0.321 0.339	0.330	0.298
175	0.604 0.634	0.619	0.587
350	1.158 1.213	1.186	1.154
700	2.123 2.204	2.164	2.132

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	20	20	20
Mean (pg/mL)	41.9	215	407	43.1	220	411
Standard deviation	2.1	6.3	8.1	2.2	11.2	18.5
CV (%)	5.0	2.9	2.0	5.1	5.1	4.5

RECOVERY

The recovery of mouse IL-17 spiked to three levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	101	92-118%
Serum (n=6)	102	91-108%
Heparin plasma (n=3)	101	91-123%
EDTA plasma (n=3)	107	93-120%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with various concentrations of mouse IL-17 in each matrix were diluted with calibrator diluent and then assayed.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=5)	Heparin plasma (n=5)
1:2	Average % of Expected	104	113	106	109
	Range (%)	101-106	107-118	98-111	107-110
1:4	Average % of Expected	98	106	108	111
	Range (%)	94-103	100-113	101-113	109-113
1:8	Average % of Expected	92	93	109	112
	Range (%)	83-97	85-104	94-119	108-114
1:16	Average % of Expected	87	86	107	109
	Range (%)	81-94	83-95	89-113	103-114

SENSITIVITY

The minimum detectable dose (MDD) of mouse IL-17 was typically less than 5 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-17 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Forty mouse serum samples and six mouse plasma samples were evaluated for detectable levels of mouse IL-17 in this assay. All samples read below the lowest standard, 10.9 pg/mL.

Cell Culture Supernates - Mouse lung conditioned media (1 lung, 1-2 mm pieces in 10 mL DMEM containing 10% fetal bovine serum and 5 µg/mL lipopolysaccharide) was collected after 6 days. The culture supernate was assayed for mouse IL-17 and measured 1800 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant mouse IL-17.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range mouse IL-17 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant mouse:

C10
Eotaxin
G-CSF
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-1ra
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-9
IL-10
IL-10 R
IL-12
IL-13
IL-17B (aa 1-180)
IL-17B (aa 21-180)
IL-17B R
IL-17D
IL-17E
IL-17F
IL-17 R
IL-18
JE/MCP-1
KC
Leptin
LIF
M-CSF
MIP-1α
MIP-1β
MIP-2
OSM
SCF
TNF-α
Tpo
VEGF

Recombinant human:

IL-17

Some cross-reactivity was observed with recombinant mouse IL-17 A/F heterodimer.

Concentration Tested (pg/mL)	Observed Value (pg/mL)	% Cross-Reactivity
100	61	61

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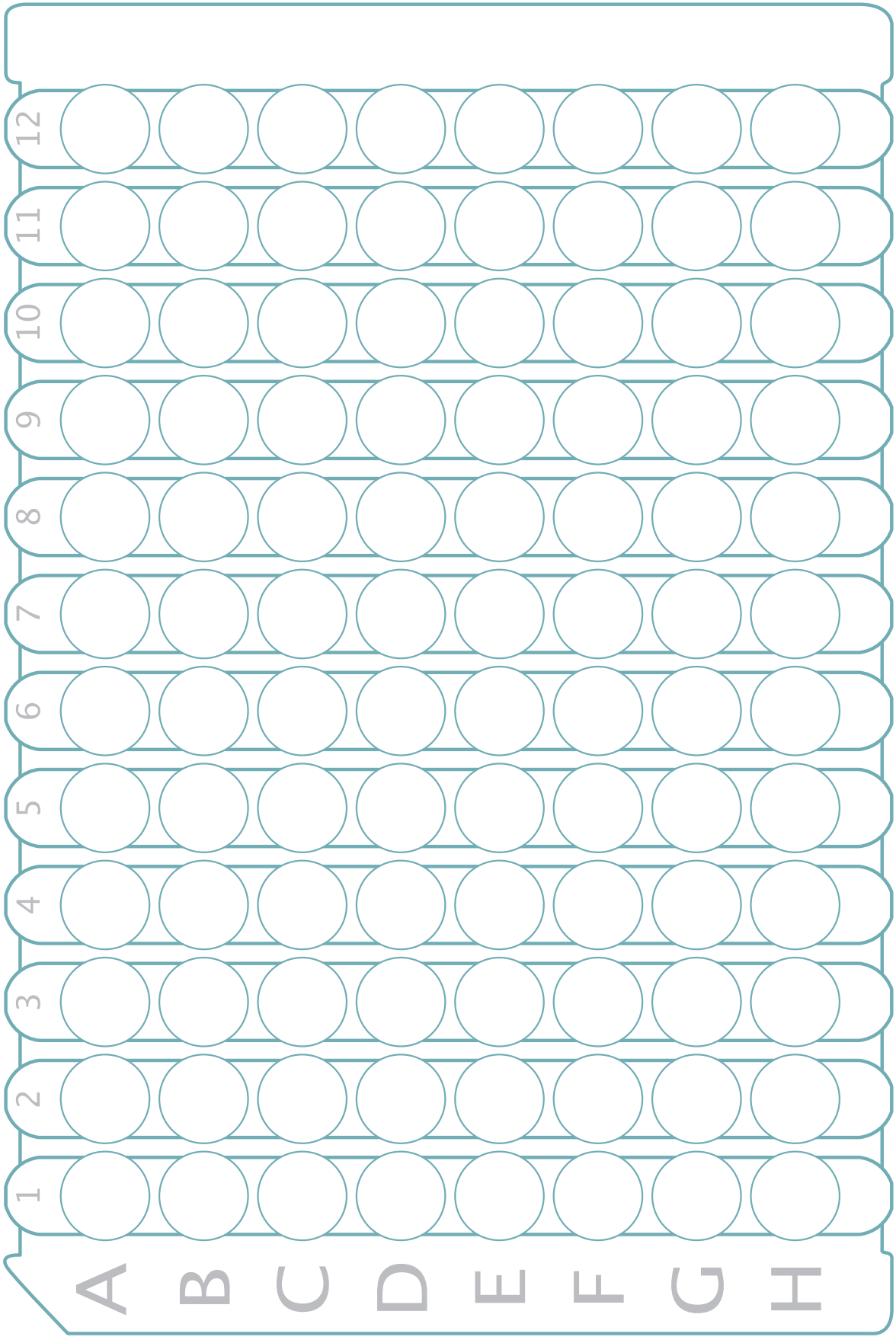
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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