Quantikine® ELISA

Canine IL-17A Immunoassay

Catalog Number CA1700

For the quantitative determination of canine Interleukin 17A (IL-17A) 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-17A (IL-17A), also known as CTLA-8, is a 15-20 kDa glycosylated cytokine that plays an important role in anti-microbial and chronic inflammation. The six IL-17 cytokines (IL-17A-F) are encoded by separate genes but adopt a conserved cystine knot fold (1, 2). IL-17A forms disulfide-linked homodimers as well as disulfide-linked heterodimers with IL-17F (5, 6). Mature canine IL-17A shares 85%, 81%, 65%, and 64% identity with feline, human, mouse, and rat IL-17A, respectively. IL-17A is secreted by Th17 cells, $CD8^+$ memory T cells, γ/δ T cells, iNKT cells, NK cells, LTi cells, neutrophils, eosinophils, monocytes and intestinal Paneth cells (2).

IL-17A exerts its effects through the transmembrane IL-17 RA in complex with IL-17 RC or IL-17 RD (7, 8). Both IL-17 RA and IL-17 RC are required for responsiveness to heterodimeric IL-17A/F (7). IL-17A promotes protective mucosal and epidermal inflammation in response to microbial infection (9-12). It induces chemokine production, neutrophil influx, and the production of antibacterial peptides (9-11). IL-17A/F likewise induces neutrophil migration, but IL-17F does not (11). IL-17A additionally enhances the production of inflammatory mediators by rheumatoid synovial fibroblasts and contributes to TNF-α induced shock (4, 13). In contrast, it can protect against the progression of colitis by limiting chronic inflammation (12). IL-17A encourages the formation of autoreactive germinal centers and exacerbates the onset and progression of experimental models of autoimmunity (14, 15). It has been shown to exert either tumorigenic or anti-tumor effects (16, 17).

The Quantikine Canine IL-17A Immunoassay is a 4.5 hour solid-phase ELISA designed to measure canine IL-17A in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant canine IL-17A and antibodies raised against the recombinant protein. Results obtained for naturally occurring canine IL-17A 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 canine IL-17A.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for canine IL-17A has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any canine IL-17A present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for canine IL-17A 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 canine IL-17A bound in the initial step. The sample values are then read from 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.
- Samples, controls, and standards must be pipetted within 15 minutes.
- 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Canine IL-17A Microplate	898110	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for canine IL-17A.	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.*
Canine IL-17A Standard	898112	2 vials of recombinant canine IL-17A 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.
Canine IL-17A Control	898113	2 vials of recombinant canine IL-17A in a buffered protein base with preservatives; lyophilized. The assay value of the Control should be within the range specified on the label.	
Canine IL-17A Conjugate	898111	12 mL of a polyclonal antibody against canine IL-17A conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-12	895214	21 mL of animal serum 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	895174	23 mL of diluted hydrochloric 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.
- 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. 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. 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 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 20 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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

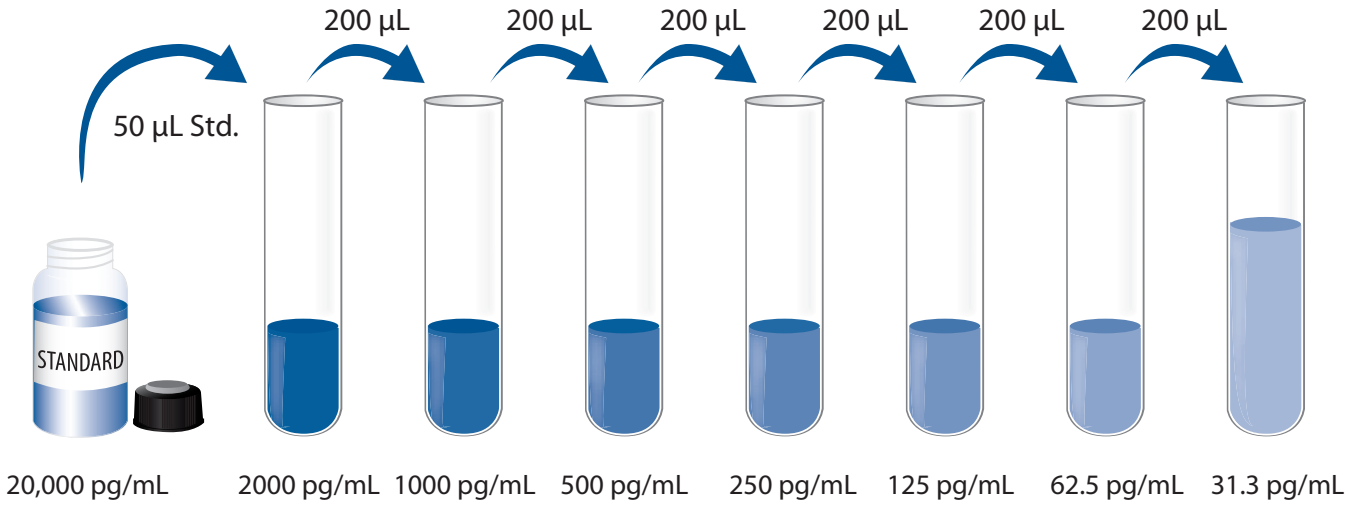
Canine IL-17A Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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 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.

Canine IL-17A Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Canine IL-17A Standard with deionized or distilled water. Do not substitute other diluents. 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 5 minutes with gentle mixing prior to making dilutions.

Pipette 450 µL of Calibrator Diluent RD6-12 into the 2000 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the standard stock solution to produce a 2-fold dilution series (below). Mix each tube thoroughly before the next transfer. The 2000 pg/mL standard serves as the high standard. Calibrator Diluent RD6-12 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, standard dilutions, Control, 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 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 ± 50 rpm. A plate layout is provided to record standards and samples assayed.

   Note: *Samples, Control, and Standards must be pipetted within 15 minutes.*
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 Canine IL-17A Conjugate to each well. Cover with a new adhesive strip. **Incubate for 2 hours at 2-8 °C without shaking. Note:** *Do not stack plates.*
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.

CALCULATION OF RESULTS

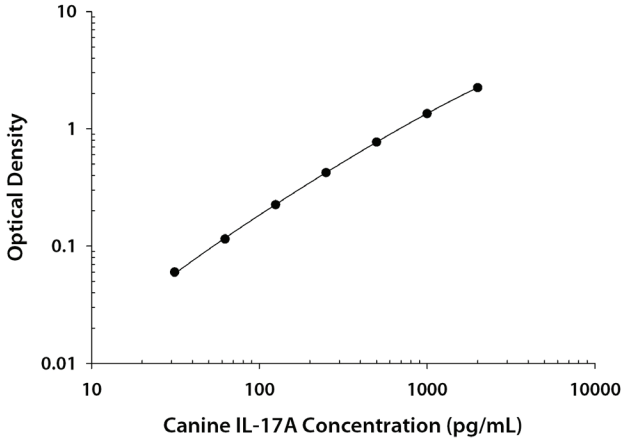
Average the duplicate readings for each standard, Control, and sample and subtract the average zero standard optical density (O.D.).

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 canine IL-17A 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	0.046 0.048	0.047	—
31.3	0.106 0.107	0.107	0.060
62.5	0.161 0.162	0.162	0.115
125	0.271 0.274	0.273	0.226
250	0.464 0.476	0.470	0.423
500	0.798 0.842	0.820	0.773
1000	1.366 1.429	1.397	1.350
2000	2.260 2.326	2.293	2.246

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)	111	257	855	107	238	797
Standard deviation	2.90	8.65	26.5	8.16	14.1	41.7
CV (%)	2.6	3.4	3.1	7.6	5.9	5.2

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	102	85-112%
Serum (n=4)	93	85-102%
EDTA plasma (n=4)	91	82-102%
Heparin plasma (n=4)	89	79-103%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of canine IL-17A were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture samples (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)
1:2	Average % of Expected	94	102	105	104
	Range (%)	87-96	99-105	99-110	101-105
1:4	Average % of Expected	90	103	109	107
	Range (%)	84-95	91-111	96-117	104-111
1:8	Average % of Expected	84	104	111	105
	Range (%)	75-95	98-114	102-122	100-109
1:16	Average % of Expected	83	105	111	103
	Range (%)	80-88	94-113	103-119	100-106

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of canine IL-17A ranged from 1.20-9.20 pg/mL. The mean MDD was 4.13 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.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed recombinant canine IL-17A produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples were evaluated for detectable levels of canine IL-17A in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=12)	ND	0	———
EDTA Plasma (n=7)	ND	0	———
Heparin Plasma (n=7)	215	14	ND-215

ND=Non-detectable

Cell Culture Supernates - Canine peripheral blood cells (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. Cell were unstimulated or stimulated with 1 µg/mL LPS or 10 ng/mL PMA + 500 ng/mL ionomycin for 24 hours. Aliquots of the cell culture supernates were removed and assayed for canine IL-17A.

Condition	(pg/mL)
Unstimulated	1452
Stimulated w/LPS	1732
Stimulated w/PMA + Ionomycin	19,536

SPECIFICITY

This assay recognizes natural and recombinant canine IL-17A.

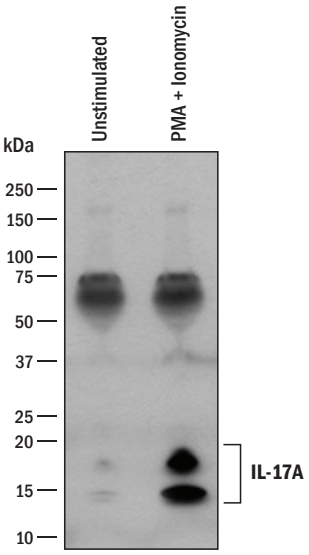
The factors listed below were prepared at 50 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range canine IL-17A control were assayed for interference. No significant cross-reactivity or interference was observed.

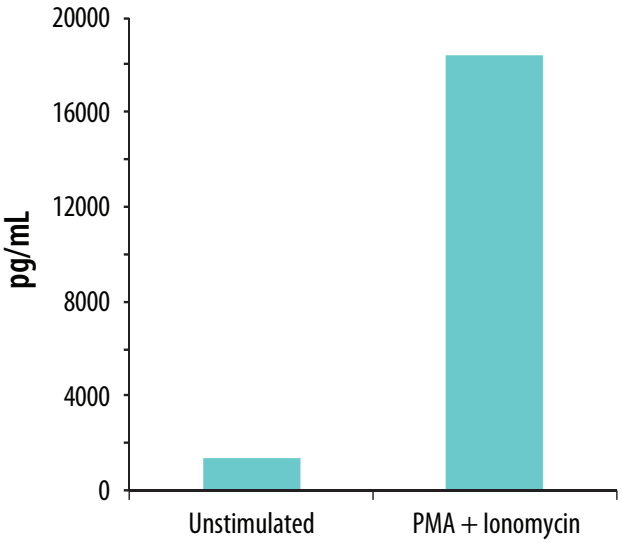
Recombinant human:
Cadherin 20
IL-4
IL-17F
IL-17 R

Recombinant mouse:
IL-17A
IL-17F

Recombinant rat:
IL-17A
IL-17F

Recombinant human IL-17 R does not cross-react but does interfere at concentrations > 10 ng/mL.





Conditioned media samples from canine PBMCs were analyzed by Western blot and Quantikine ELISA. Canine PBMCs were left unstimulated or stimulated with 10 ng/mL PMA + 500 ng/mL Ionomycin for 24 hours. Samples were resolved under reducing SDS-PAGE conditions, transferred to PVDF membrane, and immunoblotted with the detection antibody used in this kit. The Western blot shows a direct correlation with the ELISA value for these samples.

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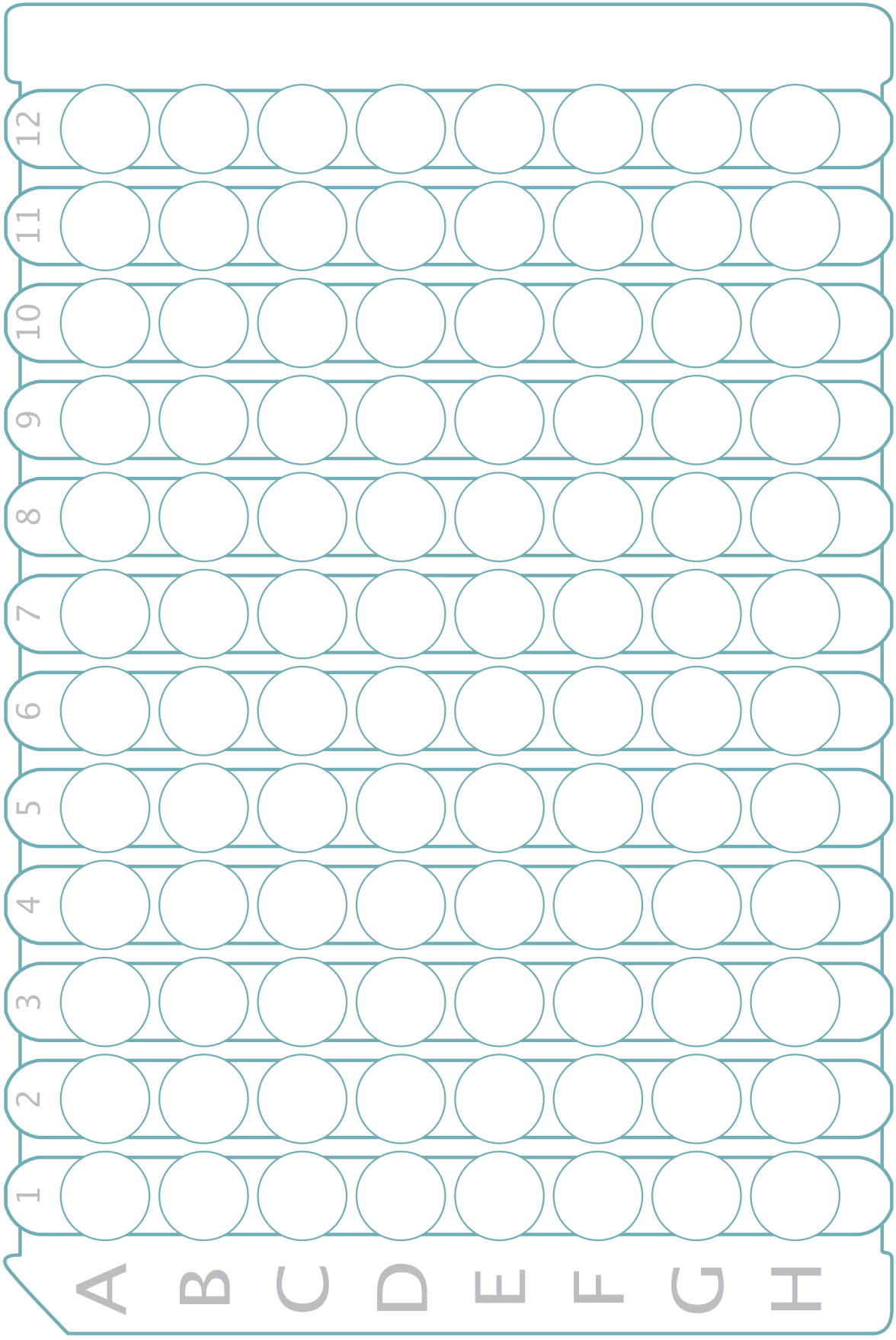
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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