Quantikine® ELISA

Canine IL-8/CXCL8 Immunoassay

Catalog Number CA8000

For the quantitative determination of canine Interleukin 8 (IL-8) 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-8 (IL-8), also known as CXCL8, GCP-1, and NAP-1, is a heparin-binding 8-9 kDa member of the alpha, or CXC family of chemokines. There are at least 15 human CXC family members that all adopt a three β-sheet/one α-helix structure. Most CXC chemokines show an N-terminal Glu-Leu-Arg (ELR) tripeptide motif (1, 2). CXCL8 circulates as a monomer, homodimer, and heterodimer with CXCL4/PF4. The monomer is considered the most bioactive, while the heterodimer can potentiate PF4 activity (3-6). CXCL8 oligomerization is modulated by its interactions with matrix and cell surface glycosaminoglycans (GAGs) (7, 8). Mature canine CXCL8 shares 87%, 69%, and 82% aa identity with feline, human, and porcine CXCL8, respectively (9, 10). There is no CXCL8 gene counterpart in rodent.

Multiple isoforms of CXCL8 are generated through differential proteolytic cleavage. Proteolytic processing results in N-terminal truncation of CXCL8 and is likely a cell-specific event. For example, fibroblasts and endothelial cells generate the 1-77 aa form by cleaving human CXCL8 following Glu21, while monocytes and lymphocytes generate the 6-77 aa form by cleaving following Leu25. These truncated forms generally show increased bioactivity, particularly through the CXCR1 receptor (11-13). CXCL8 can also undergo citrullination on Arg27 of the precursor, a modification that increases its half-life and ability to induce leukocytosis (14, 15). A wide variety of cells secrete CXCL8 including monocytes and neutrophils (16), fibroblasts and keratinocytes (17), mast cells (18), visceral smooth muscle cells (19), dendritic cells (20), type II great alveolar cells (21), and endothelial cells (22).

CXCL8 bioactivity is mediated through two G-protein-coupled receptors, termed CXCR1/IL-8 RA and CXCR2/IL-8 RB (23). CXCR1 is 45-50 kDa in size and is used almost exclusively by CXCL8. CXCR2 is 35-40 kDa in size and is used by nearly all CXC chemokines (24, 25). Both CXCR1 and CXCR2 constitutively associate into functional homodimers. They can also heterodimerize, but these complexes dissociate following CXCL8 binding (26). CXCR2 responds to low concentrations of CXCL8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, in contrast, responds to high concentrations of CXCL8 and is associated with respiratory burst and phospholipase D2 activation (25). CXCR2 ligation induces leukocyte adhesion to activated vascular endothelium and migration to sites of inflammation, while CXCR1 ligation primes neutrophil antimicrobial activity (27). CXCL8 can also form a complex with Serpin A1/ alpha-1 Antitrypsin, and this prevents CXCL8 interaction with CXCR1 (28).

In addition to its proinflammatory effects, CXCL8 is involved in angiogenesis and the pathogenesis of atherosclerosis and cancer (29-32). It induces VEGF expression in vascular endothelial cells and functions as an autocrine factor for EC growth and angiogenesis (33, 34). It is upregulated in atherosclerotic lesions and is elevated in the serum and cerebrospinal fluid following myocardial infarction (35, 36). In cancer, CXCL8 promotes epithelial-mesenchymal transition as well as tumor cell invasiveness and metastasis (31, 37-39).

The Quantikine® Canine IL-8/CXCL8 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure canine IL-8 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant canine IL-8 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural canine IL-8 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-8.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for canine IL-8 has been pre-coated onto a microplate. Standards, control, and samples are pipetted into the wells and any canine IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for canine IL-8 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-8 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 #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Canine IL-8 Microplate	893293	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for canine IL-8.	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-8 Biotin Conjugate	893294	12 mL of a monoclonal antibody specific for canine IL-8 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Canine IL-8 Standard	893295	Recombinant canine IL-8 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Canine IL-8 Control	893296	Recombinant canine IL-8 in a buffered protein base with preservatives; lyophilized. The assay value of the control should be within the range specified on the label.	
Assay Diluent RD1W	895038	12 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-26 Concentrate	895525	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:4 in this assay.*	
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.
- 100 mL and 500 mL graduated cylinders.
- 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. 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.*

SAMPLE PREPARATION

Serum and plasma samples require a 4-fold dilution. A suggested 4-fold dilution is 40 µL of sample + 120 µL of Calibrator Diluent RD5-26 (diluted 1:4)*.

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Canine IL-8 Control - Reconstitute the control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the control undiluted.

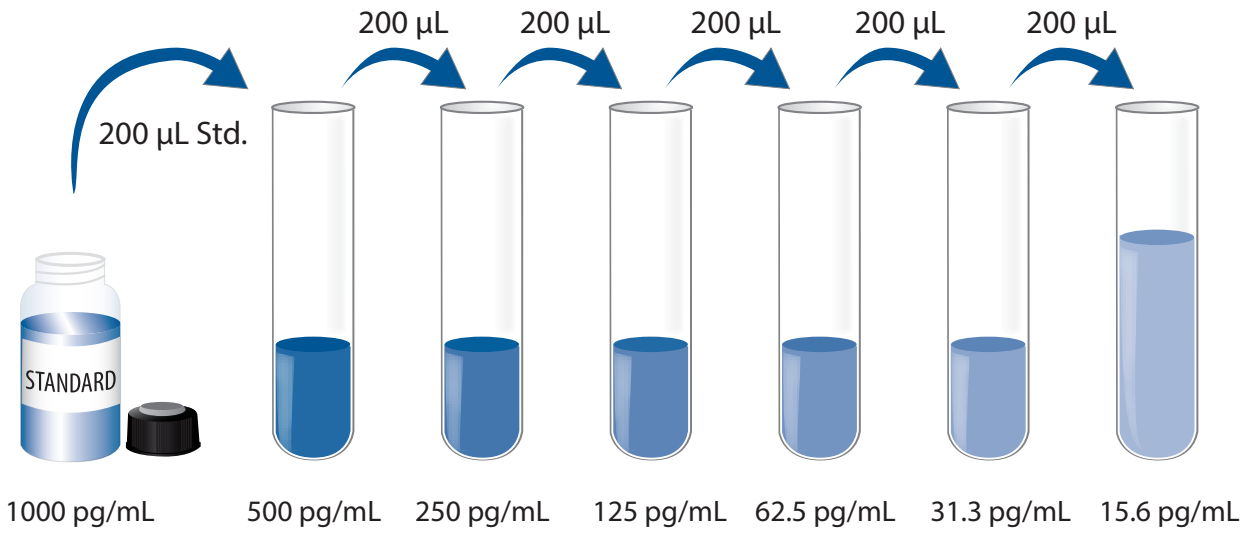
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Calibrator Diluent RD5-26 (diluted 1:4) - Add 20 mL of Calibrator Diluent RD5-26 Concentrate to 60 mL of deionized or distilled water to prepare 80 mL of Calibrator Diluent RD5-26 (diluted 1:4).

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-8 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Canine IL-8 Standard with Calibrator Diluent RD5-26 (diluted 1:4). Do not substitute other diluents. This reconstitution produces a stock solution of 1000 pg/mL. Allow the stock solution to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Pipette 200 µL of Calibrator Diluent RD5-26 (diluted 1:4) into each tube. Use the stock solution to produce a 2-fold dilution series (below). Mix each tube gently but thoroughly before the next transfer. The undiluted Canine IL-8 Standard (1000 pg/mL) serves as the high standard. Calibrator Diluent RD5-26 (diluted 1:4) 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. Tap the plate gently 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 decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Canine IL-8 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

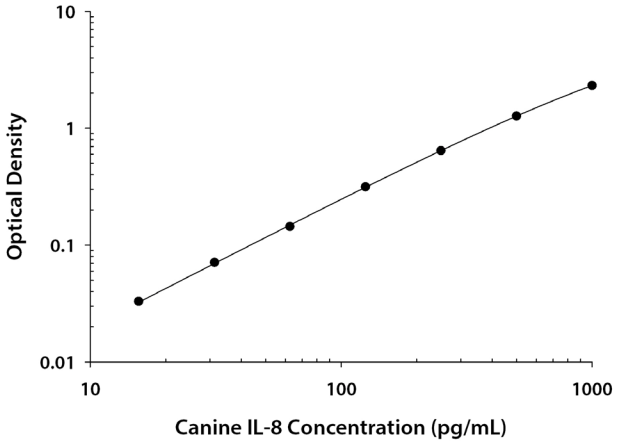
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 canine IL-8 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	0.007 0.008	0.008	—
15.6	0.040 0.042	0.041	0.033
31.3	0.078 0.079	0.079	0.071
62.5	0.150 0.153	0.152	0.144
125	0.320 0.325	0.323	0.315
250	0.643 0.659	0.651	0.643
500	1.271 1.283	1.277	1.269
1000	2.298 2.355	2.327	2.319

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 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	39	37	39
Mean (pg/mL)	38.8	105	628	43.9	116	682
Standard deviation	2.4	5.8	32.4	2.6	5.5	36.1
CV (%)	6.2	5.5	5.2	5.9	4.7	5.3

RECOVERY

The recovery of canine IL-8 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=6)	97	88-103%
Serum* (n=4)	97	91-101%
EDTA plasma* (n=4)	100	93-108%
Heparin plasma* (n=4)	102	91-113%

*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 high concentrations of canine IL-8 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=4)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	100	101	103	102
	Range (%)	91-107	93-104	101-105	99-108
1:4	Average % of Expected	99	107	105	106
	Range (%)	87-104	100-116	100-108	102-113
1:8	Average % of Expected	100	110	111	110
	Range (%)	89-107	106-114	107-114	105-118
1:16	Average % of Expected	105	109	112	113
	Range (%)	91-113	103-119	107-118	108-119

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

SENSITIVITY

Thirty-six assays were evaluated and the minimum detectable dose (MDD) of canine IL-8 ranged from 0.49-4.31 pg/mL. The mean MDD was 1.26 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 canine IL-8 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma -Samples were evaluated for detectable levels of canine IL-8 in this assay.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=17)	1307	88	ND-2908
EDTA plasma (n=15)	376	87	ND-1477
Heparin plasma (n=19)	414	83	ND-1430

ND=Non-detectable

Cell Culture Supernates:

Canine peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 1% HEPES, 50 µM β-mercaptoethanol, 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 and 10 µM histamine for 2 days. An aliquot of the cell culture supernate was removed, assayed for canine IL-8, and measured 44.1 ng/mL.

Canine peripheral blood leukocytes (6 x 10^5 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum, 10 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. Cells were cultured unstimulated or stimulated with 10 µg/mL Con A for 4 days. An aliquot of the cell culture supernate was removed, assayed for canine IL-8, and measured 10.2 ng/mL.

A-72 canine fibroma cells (3 x 10^5 cells/mL) were cultured in Leibovitz's L-15 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 3 days. An aliquot of the cell culture supernate was removed, assayed for canine IL-8, and measured 19.7 ng/mL.

GGE canine gallbladder epithelial cells (1 x 10^5 cells/mL) were cultured in RPMI with 10% fetal bovine serum, 2 mM L-glutamine, and 50 ng/mL of recombinant canine IL-6. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 3 days. An aliquot of the cell culture supernate was removed, assayed for canine IL-8, and measured 102.8 ng/mL.

MDCK canine kidney epithelial cells (1 x 10^5 cells/mL) were cultured in RPMI supplemented with 10% fetal bovine serum and stimulated with 10 µg/mL PHA and 10 ng/mL PMA for 3 days. An aliquot of the cell culture supernate was removed, assayed for canine IL-8, and measured 801 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant canine IL-8 (74 and 79 aa isoforms).

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-8 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant canine:

IL-2
IL-4
IL-5
IL-6
IL-10
IL-12/IL-23 p40
IL-18
MCP-1

Recombinant human:

GROα
GROγ
IL-8
IP-10
MCP-1
MCP-2
MIP-1β
MIP-1δ
PF-4

Recombinant porcine:

IL-6
IL-8
IL-12

Other recombinants:

feline IL-8
mouse KC

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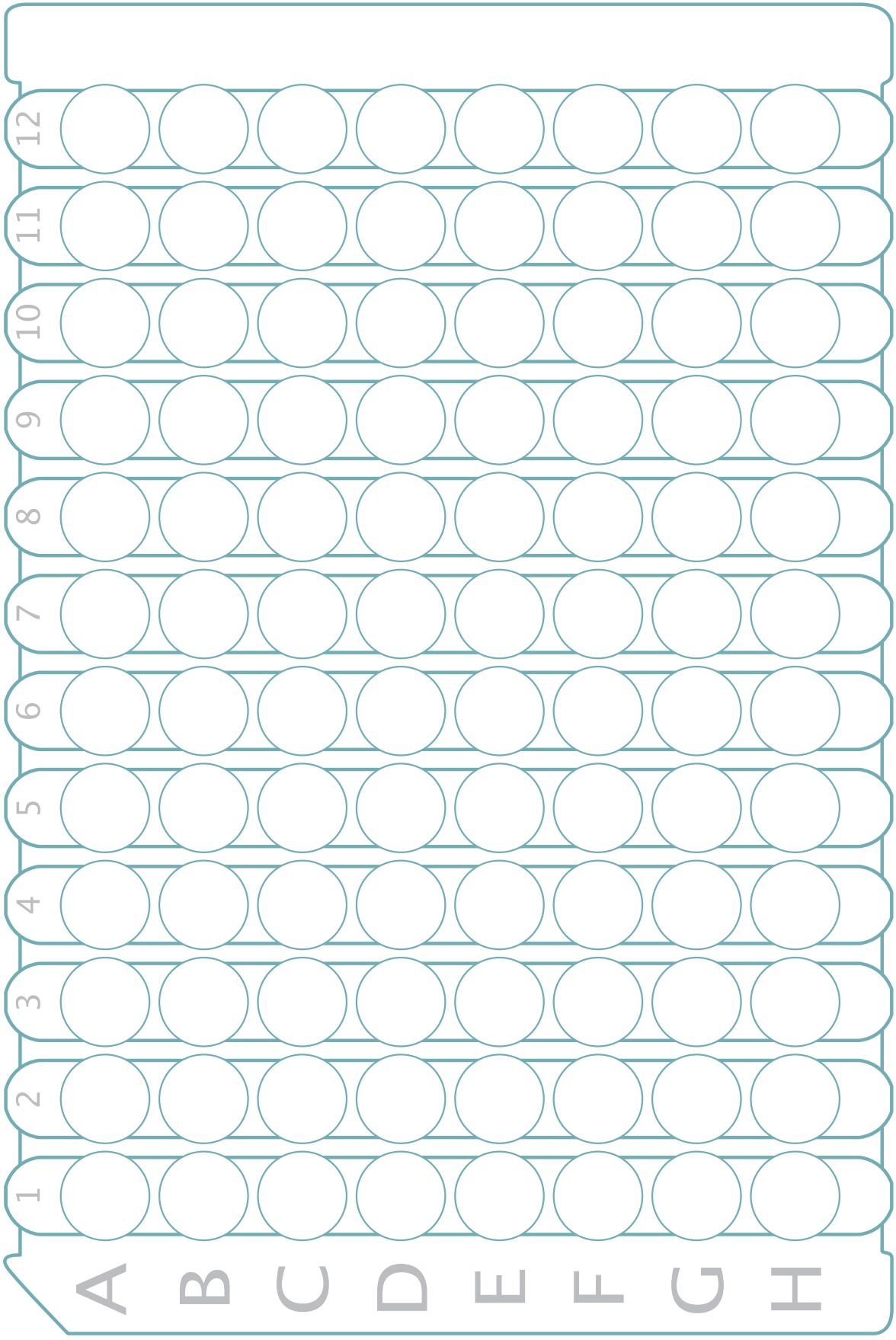
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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