Quantikine® ELISA

Rat CXCL1/CINC-1 Immunoassay

Catalog Number RCN100

For the quantitative determination of rat Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1) concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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

Rat Cytokine-Induced Neutrophil Chemoattractant-1 (CINC-1), otherwise known as CXCL1, CINC, rat GROα, and rat KC, is an 8 kDa member of the α-, or CXC family of chemokines (1-4). It is synthesized as a 96 amino acid (aa) precursor that contains a 24 aa signal sequence plus a 72 aa mature segment (5, 6). In the mature segment, there are two intrachain disulfide bonds plus an ELR and CXC motif. There are no potential N-linked glycosylation sites. The molecule reportedly circulates as a nondisulfide-linked homodimer (1, 5, 7). In the mature region, rat CINC-1 shows 66% and 70% aa sequence identity to rat CINC-2α and CINC-3, respectively (1, 5, 8, 9). It shows 92% aa sequence identity to mouse KC and 69% aa sequence identity to human GROα (5, 10). Cells known to express CINC-1 are varied and include hepatocytes (11, 12), mast cells (13), macrophages (14-16), neurons (17), Kupffer cells (12), fibroblasts (18), type II greater alveolar cells (19), and cardiac muscle cells (20).

The receptor for rat CINC-1 is CXCR2 (21, 22). Although both rat CXCR1-like and CXCR2 have been reported in the literature (22), the CXCR1-like molecule is unresponsive to CXC ligands. Thus, only CXCR2 is considered the functional CINC receptor (21).

Functionally, CINC-1 is described as a major neutrophil chemoattractant and activator (1, 13, 23-25). CINC-1, induced by IL-1β, TNF-α and bacterial products, promotes both neutrophil rolling and adhesion, likely through the upregulation of surface integrins (15, 25-28). Thus, it directs neutrophils to sites of bacterially-induced inflammation (27). It is also reported to stimulate neutrophil activity by promoting cathepsin G release from azurophilic granules (23). It does not, however, seem to impact nitrite production (24). Thus it makes a partial contribution to bacterial killing. Relative to CINC-2α and CINC-3, CINC-1 seems to be equal in chemotactic activity but less efficient in inducing calcium mobilization (29). It is also induced earlier in macrophages than CINC-2 and -3 and declines more quickly in expression (16). The significance of this is unclear.

The Quantikine Rat CXCL1/CINC-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure rat CINC-1 in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant rat CINC-1 and antibodies raised against the recombinant factor. This immunoassay has been shown to accurately quantitate the recombinant factor. Results obtained using natural rat CINC-1 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 naturally occurring rat CINC-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A polyclonal antibody specific for rat CINC-1 has been pre-coated onto a microplate. Standards, Control, and samples are pipetted into the wells and any rat CINC-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for rat CINC-1 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 rat CINC-1 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
Rat CINC-1 Microplate	892696	96 well polystyrene microplate (12 strips of 8 wells) coated with a polyclonal antibody specific for rat CINC-1.	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.*
Rat CINC-1 Conjugate	892697	12 mL of a polyclonal antibody specific for rat CINC-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Rat CINC-1 Standard	892698	Recombinant rat CINC-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Rat CINC-1 Control	892699	Recombinant rat CINC-1 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 RD5-4	895435	21 mL of a buffered protein solution 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.
- **Polypropylene** 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.*
Grossly hemolyzed or lipemic samples may not be suitable for use in this assay.

SAMPLE PREPARATION

Serum and plasma samples require a 2-fold dilution prior to assay. A suggested 2-fold dilution is 70 µL of sample + 70 µL of Calibrator Diluent RD5-4.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

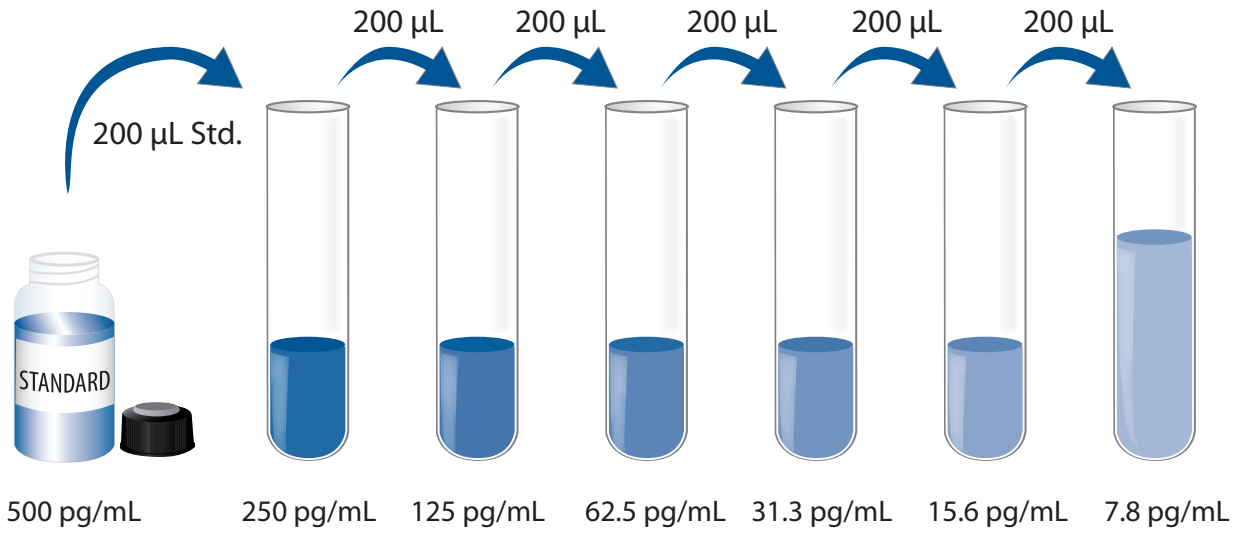
Rat CINC-1 Control - Reconstitute the Control with 1.0 mL of deionized or distilled water. Mix thoroughly. Assay the Control undiluted.

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.

Rat CINC-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Rat CINC-1 Standard with Calibrator Diluent RD5-4. Do not substitute other diluents. This reconstitution produces a stock solution of 500 pg/mL. Allow the standard to sit for a minimum of 5 minutes with gentle mixing prior to making dilutions.

Use polypropylene tubes. Pipette 200 µL of Calibrator Diluent RD5-4 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 Rat CINC-1 Standard (500 pg/mL) serves as the high standard. Calibrator Diluent RD5-4 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. 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 decanting. Invert the plate and blot it against clean paper towels.
6. Add 100 µL of Rat CINC-1 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

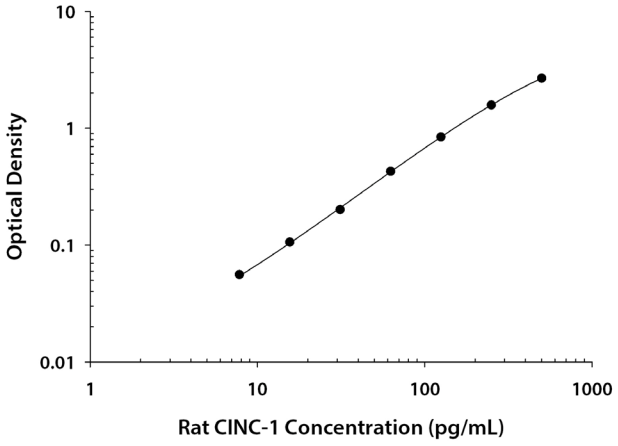
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 rat CINC-1 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	0.038 0.046	0.042	—
7.8	0.097 0.099	0.098	0.056
15.6	0.146 0.150	0.148	0.106
31.3	0.240 0.246	0.243	0.201
62.5	0.466 0.472	0.469	0.427
125	0.871 0.896	0.884	0.842
250	1.593 1.648	1.620	1.578
500	2.690 2.749	2.720	2.678

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-three 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	23	23	23
Mean (pg/mL)	53	71	215	49	71	190
Standard deviation	2.7	5.2	12	3.1	3.3	12
CV (%)	5.1	7.3	5.6	6.3	4.6	6.3

RECOVERY

The recovery of rat CINC-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture supernates (n=4)	99	93-106
Serum* (n=5)	95	89-103
EDTA plasma* (n=4)	97	90-104
Heparin plasma* (n=4)	99	90-113

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of rat CINC-1 were diluted with Calibrator Diluent and assayed.

		Cell culture supernates (n=5)	Serum* (n=4)	EDTA plasma* (n=4)	Heparin plasma* (n=4)
1:2	Average % of Expected	103	103	103	105
	Range (%)	97-114	101-104	100-105	102-108
1:4	Average % of Expected	101	102	105	109
	Range (%)	94-110	97-106	99-110	105-112
1:8	Average % of Expected	99	106	105	110
	Range (%)	93-108	103-109	99-110	101-114
1:16	Average % of Expected	101	109	112	108
	Range (%)	94-105	103-114	109-117	98-118

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

SENSITIVITY

Twelve assays were evaluated and the minimum detectable dose (MDD) of rat CINC-1 ranged from 0.7-1.3 pg/mL. The mean MDD was 1.1 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 rat CINC-1 produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma -Samples were evaluated for the presence of rat CINC-1 in this assay.

Sample	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=20)	212	95-579	114
EDTA plasma (n=20)	46	16-110	21
Heparin plasma (n=4)	145	54-252	83

Cell Culture Supernates:

Rat spleen, cut into 1-2 mm pieces, was cultured for 3 days in 50 mL DMEM supplemented with 10% fetal calf serum and stimulated with 100 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for rat CINC-1, and measured 3166 pg/mL.

Rat lung, cut into 1-2 mm pieces, was cultured for 3 days in 50 mL DMEM supplemented with 10% fetal calf serum and stimulated with 100 ng/mL LPS. An aliquot of the cell culture supernate was removed, assayed for rat CINC-1, and measured 1040 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant rat CINC-1.

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 rat CINC-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant rat:

β-NGF
C-Ag.3.4.8
CINC-3
CNTF
CNTF Rα
E-Selectin
EphA5
EphB1
Fractalkine
GDNF
GDNF Rα1
GM-CSF
IFN-γ
IL-1 R6
IL-1ra
IL-1α
IL-1β
IL-2
IL-4
IL-6
IL-10
IL-18
LIX
MAG
MIP-3α
Npn-1
Npn-2
PDGF-AA
PDGF-AB
PDGF-BB
TIMP-1
TNF-α
VEGF

Recombinant mouse:

MIP-2

Recombinant human:

GROα
GROβ
GROγ

Recombinant rat CINC-2α and recombinant rat CINC-2β cross-react approximately 0.04% in this assay.

Recombinant mouse KC cross-reacts approximately 20% in this assay.

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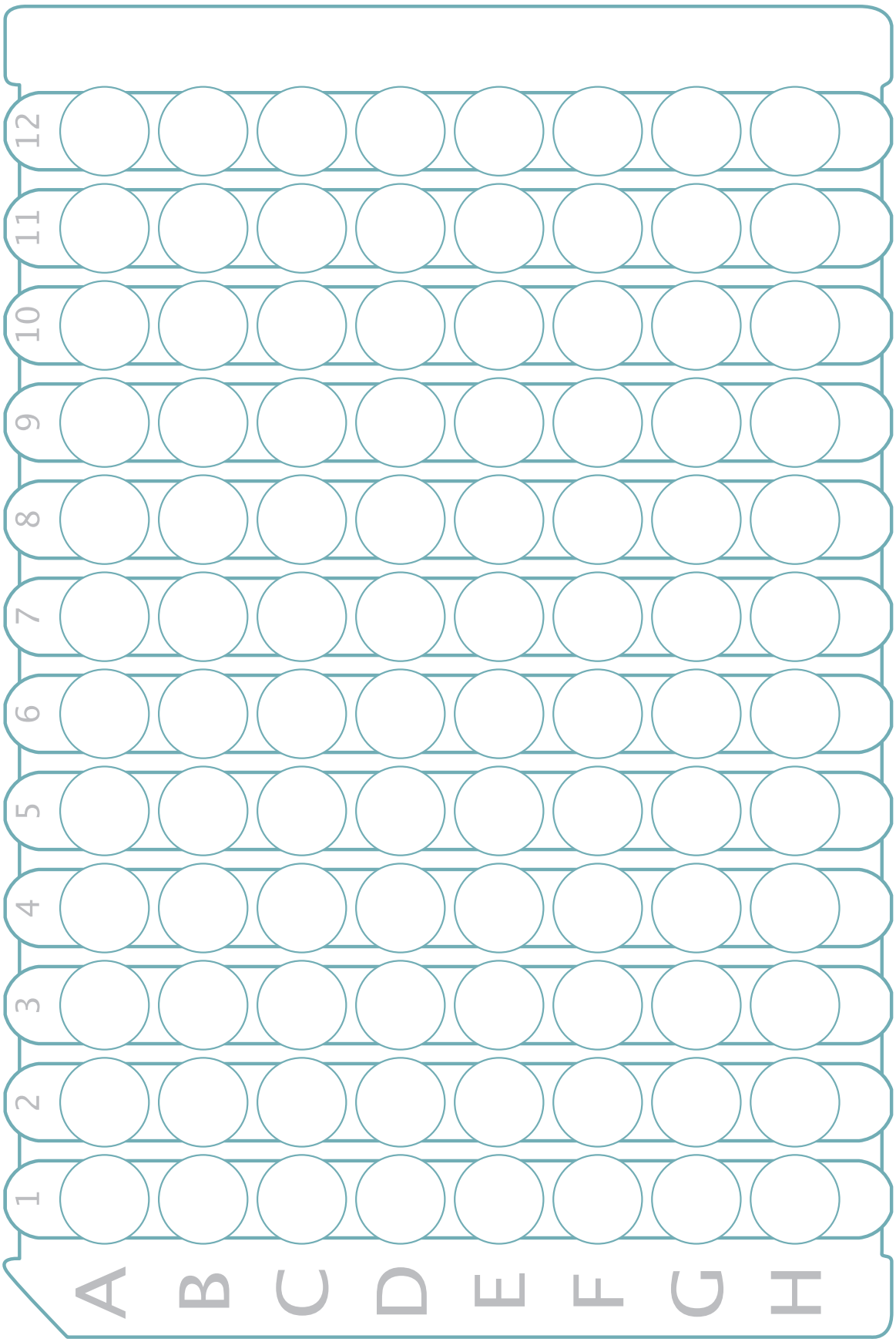
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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