QuantiGlo® ELISA

Human CXCL8/IL-8 Immunoassay

Catalog Number Q8000B

For the quantitative determination of human 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.

TABLE OF CONTENTS

SECTION	PAGE
INTRODUCTION	1
PRINCIPLE OF THE ASSAY	2
TECHNICAL HINTS & LIMITATIONS	2
MATERIALS PROVIDED & STORAGE CONDITIONS	3
OTHER SUPPLIES REQUIRED	4
SAMPLE COLLECTION & STORAGE	4
REAGENT PREPARATION	5
ASSAY PROCEDURE	6
CALCULATION OF RESULTS	7
TYPICAL DATA	7
PRECISION	8
RECOVERY	8
SENSITIVITY	8
CALIBRATION	8
LINEARITY	9
SAMPLES VALUES	9
SPECIFICITY	10
REFERENCES	11
PLATE LAYOUT	12

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INTRODUCTION

Interleukin-8 (IL-8), also known as IL-8, 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). IL-8 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). IL-8 oligomerization is modulated by its interactions with matrix and cell surface glycosaminoglycans (GAGs) (7, 8). Mature human IL-8 shares 65-69% amino acid (aa) identity with canine, feline, and porcine IL-8 (9, 10). There is no IL-8 gene counterpart in rodent.

Multiple isoforms of IL-8 are generated through both alternative splicing and differential proteolytic cleavage. In humans, alternative splicing generates an isoform with an eleven aa substitution at the C-terminus (11). Proteolytic processing results in N-terminal truncation of IL-8 and is likely a cell-specific event. For example, fibroblasts and endothelial cells generate the 1-77 aa form by cleaving IL-8 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 (12-14). IL-8 can also undergo citrullination on Arg27 of the precursor, a modification that increases its half-life and ability to induce leukocytosis (15, 16). A wide variety of cells secrete IL-8 including monocytes and neutrophils (17), fibroblasts and keratinocytes (18), mast cells (19), visceral smooth muscle cells (20), dendritic cells (21), type II great alveolar cells (22), and endothelial cells (23).

IL-8 bioactivity is mediated through two G-protein-coupled receptors, termed CXCR1/IL-8 RA and CXCR2/IL-8 RB (24). CXCR1 is 45-50 kDa in size and is used almost exclusively by IL-8. CXCR2 is 35-40 kDa in size and is used by nearly all CXC chemokines (25, 26). Both CXCR1 and CXCR2 constitutively associate into functional homodimers. They can also heterodimerize, but these complexes dissociate following IL-8 binding (27). CXCR2 responds to low concentrations of IL-8 and is principally associated with chemotaxis and MMP-9 release. CXCR1, in contrast, responds to high concentrations of IL-8 and is associated with respiratory burst and phospholipase D2 activation (26). CXCR2 ligation induces leukocyte adhesion to activated vascular endothelium and migration to sites of inflammation, while CXCR1 ligation primes neutrophil antimicrobial activity (28). IL-8 can also form a complex with Serpin A1/alpha-1 Antitrypsin, and this prevents IL-8 interaction with CXCR1 (29).

In addition to its proinflammatory effects, IL-8 is involved in angiogenesis and the pathogenesis of atherosclerosis and cancer (30-33). It induces VEGF expression in vascular endothelial cells and functions as an autocrine factor for EC growth and angiogenesis (34, 35). It is upregulated in atherosclerotic lesions and is elevated in the serum and cerebrospinal fluid following myocardial infarction (36, 37). In cancer, IL-8 promotes epithelial-mesenchymal transition as well as tumor cell invasiveness and metastasis (32, 38-40).

The QuantiGlo Human CXCL8/IL-8 Immunoassay is a 5 hour solid phase ELISA designed to measure human IL-8 levels in cell culture supernates, serum, and plasma. It contains the 72 amino acid variant of human IL-8 derived from *E. coli* as well as antibodies raised against this recombinant factor. This immunoassay has been shown to accurately quantitate recombinant human IL-8. Results obtained using natural human IL-8 showed linear curves that were parallel to the standard curves obtained using the QuantiGlo kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IL-8.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human IL-8 is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, an enhanced luminol/peroxide substrate solution is added to the wells and light is produced in proportion to the amount of IL-8 bound in the initial step. A microplate luminometer is used to measure the intensity of the light emitted.

TECHNICAL HINTS & LIMITATIONS

- 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.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the QuantiGlo Immunoassay, the possibility of interference cannot be excluded.
- Variations in pipetting technique, washing technique, luminometers, incubation time or temperature can cause variations in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- 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.
- Relative light units (RLUs) may differ among luminometers. Adjust settings as recommended by the instrument manufacturer.
- Relative light units may vary within the 20 minute reading window.
- Due to limitations of many luminometers, it is recommended that the standards be assayed in duplicate from high to low beginning with the high standard in wells A_1 and A_2.

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
Human IL-8 Microplate	892813	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human 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.*
Human IL-8 Conjugate	892841	21 mL of a polyclonal antibody specific for human IL-8 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human IL-8 Standard	890578	12.5 ng of recombinant human IL-8 in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-86	895878	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6P	895118	21 mL of animal serum with preservatives.	
Wash Buffer Concentrate	895222	100 mL of a 10-fold concentrated solution of buffered surfactant with preservatives.	
Glo Reagent A	895868	12 mL of stabilized enhanced luminol.	
Glo Reagent B	895869	12 mL of stabilized hydrogen peroxide.	
Plate Sealers	N/A	4 adhesive strips.	

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

OTHER SUPPLIES REQUIRED

- Luminometer set with the following parameters: 1.0 minute lag time; 1 sec/well read time; summation mode; auto gain on, or equivalent.
- Pipettes and pipette tips.
- 1000 mL graduated cylinder.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards.
- Human IL-8 Controls (optional; R&D Systems, Catalog # QC194).

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 - Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 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, heparin, or citrate as an anticoagulant. Centrifuge for 15 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: *Hemolyzed samples are not suitable for use in this assay.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

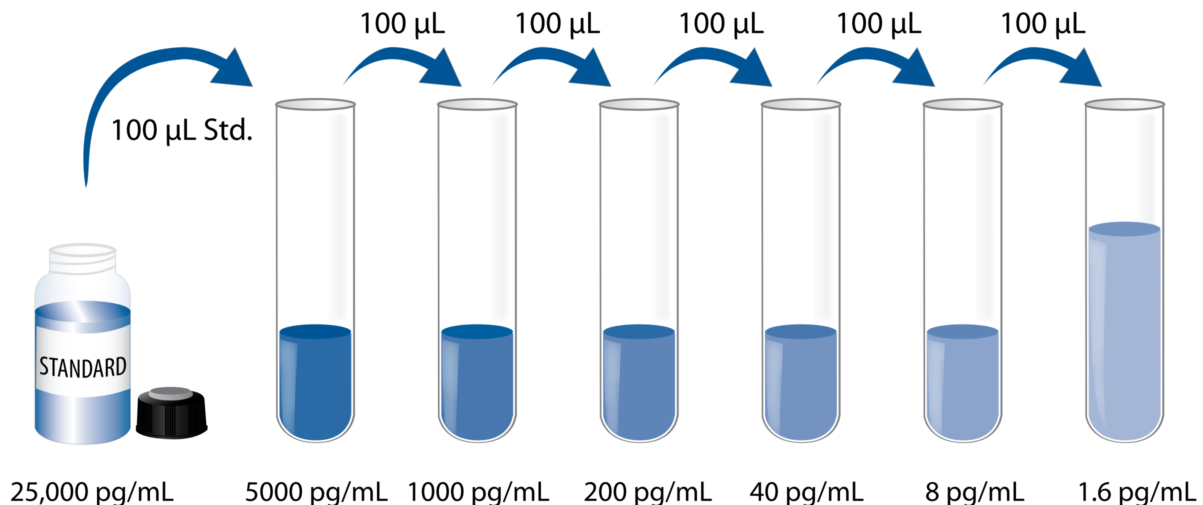
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 100 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 1000 mL of Wash Buffer.

Working Glo Reagent - 1 part Glo Reagent A (4 mL) and 2 parts Glo Reagent B (8 mL) should be mixed together 15 minutes to 4 hours before use in a capped plastic container and protected from light. 100 μL of the resultant mixture is required per well.

Note: *If running the assay in less than 96 wells, mix appropriate amounts Glo Reagent A and Glo Reagent B. To assay half a plate (48 wells), mix 2 mL of Glo Reagent A with 4 mL of Glo Reagent B. Working Glo Reagent should be discarded after use.*

Human IL-8 Standard - Reconstitute the Human IL-8 Standard with 0.5 mL of deionized or distilled water. This reconstitution produces a stock solution of 25,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 400 μL of Calibrator Diluent RD6P into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 5000 pg/mL standard serves as the high standard. Calibrator Diluent RD6P 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, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, 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 100 µL of Assay Diluent RD1-86 to each well.
4. Add 50 µL of Standard, sample, or control 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 the 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 200 µL of Human IL-8 Conjugate to each well. Cover with a new adhesive strip. Incubate for 3 hours at room temperature on the shaker.

   Note: *Prepare Working Glo Reagent at this time.*
7. Repeat the aspiration/wash as in step 5.
8. Add 100 µL of Working Glo Reagent to each well. Incubate for 5-20 minutes at room temperature **on the benchtop. Protect from light.**
9. Determine the RLU of each well using a luminometer set with the following parameters; 1.0 min. lag time; 1 sec/well read time; summation mode; auto gain on, or equivalent.

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CALCULATION OF RESULTS

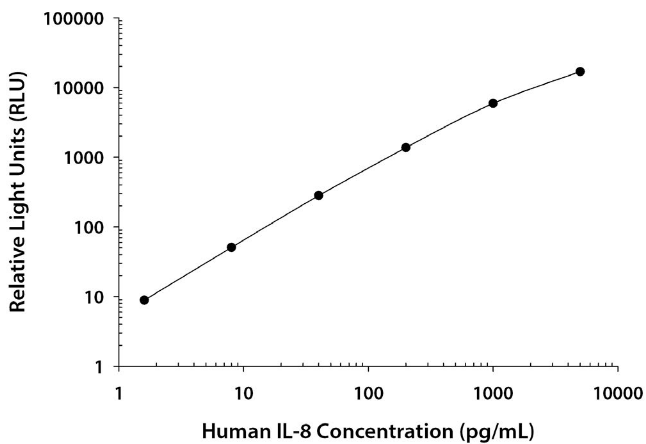
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard RLU.

Create a standard curve by reducing the data using computer software capable of generating a cubic-spline curve fit.

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)	RLU	Average	Corrected
0	9.17 9.93	9.55	—
1.6	18.4 18.5	18.4	8.85
8	58.3 62.2	60.2	50.7
40	288 293	290	280
200	1356 1404	1380	1370
1000	5820 6000	5910	5900
5000	16,420 17,391	16,905	16,895

PRECISION

Intra-assay Precision (Precision within an assay)
Four samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Four 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	4	1	2	3	4
n	20	20	20	20	20	20	20	20
Mean (pg/mL)	3.66	22.5	228	2263	4.19	24.9	243	2393
Standard deviation	0.241	1.02	8.98	146	0.481	1.57	14.7	216
CV (%)	6.6	4.5	3.9	6.5	11.5	6.3	6.0	9.0

RECOVERY

The recovery of natural and recombinant human IL-8 spiked to three different levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	98-107%
Serum (n=4)	103	93-107%
EDTA plasma (n=4)	105	95-113%
Heparin plasma (n=4)	107	102-113%
Citrate plasma (n=4)	101	97-107%

SENSITIVITY

Twenty-one assays were evaluated and the minimum detectable dose (MDD) of human IL-8 ranged from 0.12-0.97 pg/mL. The mean MDD was 0.28 pg/mL.

The MDD was determined by adding two standard deviations to the mean RLU of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified *E. coli*-expressed 72 amino acid (monocyte) form of recombinant human IL-8 produced at R&D Systems.

The NIBSC/WHO 1st International Standard 89/520 was evaluated in this kit. The dose response curve of this standard parallels the QuantiGlo standard curve. To convert sample values obtained with the QuantiGlo Human IL-8 kit to approximate NIBSC International units, use the equation below.

NIBSC (89/520) approximate value (IU/mL) = 0.001 x QuantiGlo Human IL-8 value (pg/mL)

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human IL-8 were diluted with the appropriate Calibrator Diluent to produce samples with values within the dynamic range of the assay.

		Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Citrate plasma (n=4)
1:2	Average % of Expected	103	103	99	101	94
	Range (%)	101-104	97-108	94-102	97-105	90-98
1:4	Average % of Expected	103	101	96	100	94
	Range (%)	100-105	96-104	91-98	94-109	90-96
1:8	Average % of Expected	108	105	98	104	96
	Range (%)	107-109	98-113	94-103	100-109	93-102
1:16	Average % of Expected	108	100	96	102	91
	Range (%)	105-109	90-108	90-102	99-104	84-102
1:32	Average % of Expected	105	96	94	99	90
	Range (%)	102-109	85-109	91-98	96-101	81-103
1:64	Average % of Expected	106	97	93	100	91
	Range (%)	100-113	86-107	87-98	89-109	87-96

SAMPLES VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human IL-8 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=37)	11.8	3.23-24.5	4.47
EDTA plasma (n=37)	3.79	1.75-7.74	1.51
Heparin plasma (n=37)	4.51	1.87-9.55	2.10
Citrate plasma (n=20)	3.30	1.78-13.26	1.18

Cell Culture Supernates - Human peripheral blood mononuclear cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% fetal calf serum, 50 μM β-mercaptoethanol, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin sulfate and stimulated with 10 μg/mL PHA. Aliquots of the cell culture supernates were removed on days 1 and 6 and assayed for levels of natural human IL-8.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	15,171	94,267
Stimulated	29,368	488,891

SPECIFICITY

This assay recognizes natural and recombinant human IL-8.

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

Recombinant human:

ENA-78
BLC/BCA-1
GCP-2
GROα
GROβ
GROγ
IP-10
I-TAC
MIG
NAP-2
PF4
SDF-1α
SDF-1β

Recombinant mouse:

BLC/BCA-1
IP-10/CRG-2
GCP-2
KC
MIG
SDF-1α

Recombinant canine:

IL-8

Recombinant porcine IL-8 cross-reacts approximately 0.17% in this assay.

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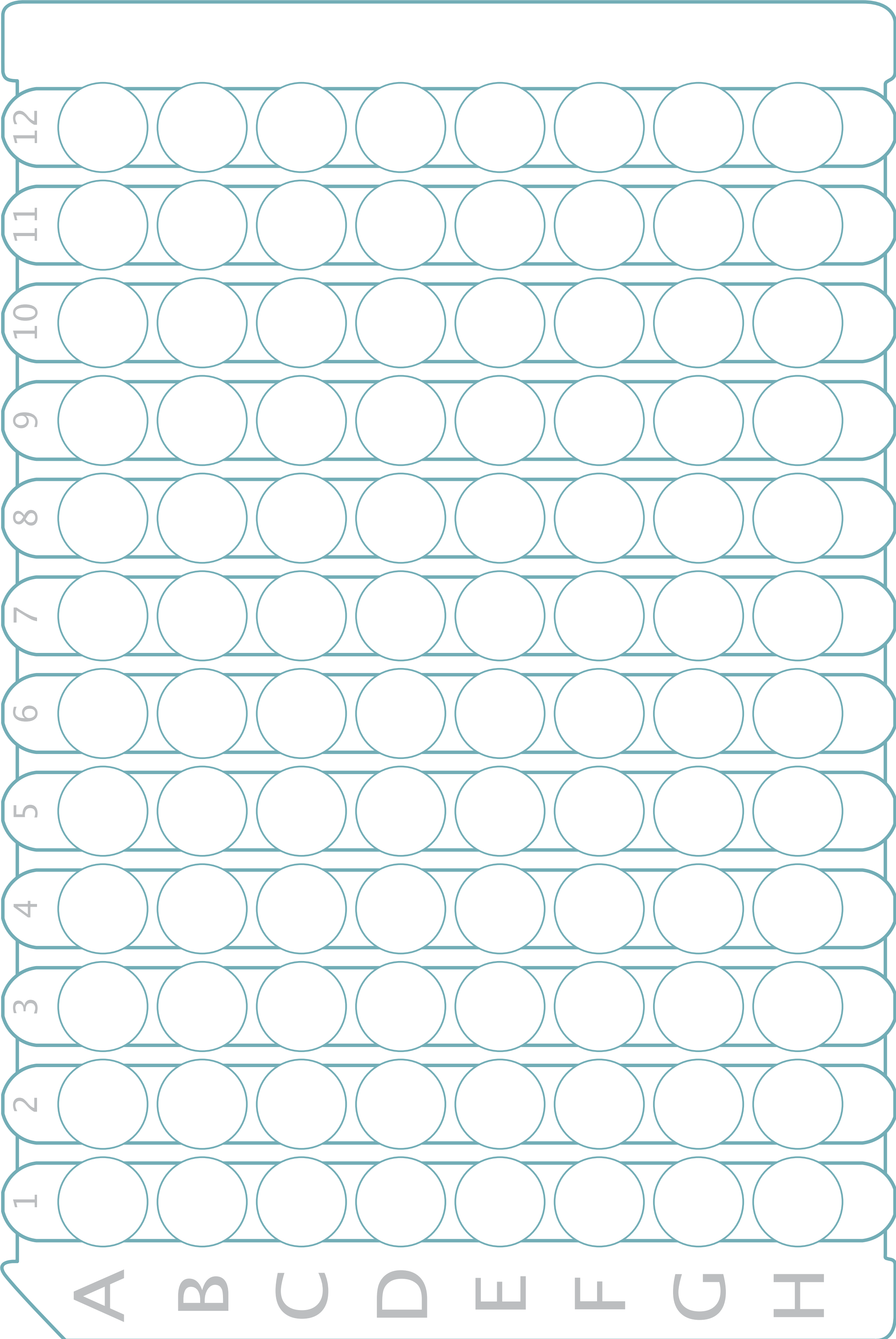
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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