Quantikine® QuicKit™ ELISA

Human G-CSF Immunoassay

Catalog Number QK214

For the quantitative determination of human Granulocyte Colony Stimulating Factor (G-CSF) 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

Granulocyte-colony stimulating factor (G-CSF) is a 24-25 kDa monomeric glycoprotein that regulates the proliferation, differentiation, and activation of hematopoietic cells in the neutrophilic granulocyte lineage (1, 2). Mature human G-CSF is a 178 amino acid (aa) O-glycosylated protein that contains two intrachain disulfide bridges (3, 4). In humans, alternate splicing generates a second minor isoform with a 3 aa deletion (5). Mouse and human G-CSF share 76% aa sequence identity, and the two proteins show species cross-reactivity (4, 6, 7). G-CSF is produced by activated monocytes and macrophages, fibroblasts, endothelial cells, astrocytes, neurons, and bone marrow stroma cells (8-10). In addition, various tumor cells express G-CSF constitutively (11).

Human G-CSF receptor (G-CSF R) is a 120 kDa type I transmembrane glycoprotein that belongs to the hematopoietin receptor superfamily (7, 12). The mature protein consists of a 603 aa extracellular domain (ECD), a 23 aa transmembrane segment, and a 186 aa cytoplasmic domain (12). The ECD contains an N-terminal Ig-like domain, a cytokine receptor homology domain, and three fibronectin type III domains (12-14). Alternate splicing of human G-CSF R generates additional isoforms including a potentially soluble form of the receptor (7). The ECDs of mouse and human G-CSF R share 63% aa sequence identity (7, 9). G-CSF R forms a complex with the ligand in a 2:2 ratio (13-15). It is expressed on monocytes, neutrophils, megakaryocytes, platelets, myeloid progenitors, trophoblasts and placenta, endothelial cells, and various tumor cell types (11, 16-20).

G-CSF is an important regulator for granulopoiesis *in vivo*, and mutations in G-CSF R are associated with congenital neutropenia (1, 21). G-CSF can support the growth of multi-lineage hematopoietic progenitor cells and mobilize them from the bone marrow into the bloodstream (22-26). G-CSF enhances the functional capacity of mature neutrophils and supports their survival by limiting the rate of apoptosis (25, 27-29). G-CSF also enhances M-CSF induced monocytopoiesis from hematopoietic progenitor cells and stimulates the proliferation of peripheral Th2-inducing dendritic cells (30, 31). It promotes the development of T cell immune tolerance as well as tissue recovery following myocardial infarction and cerebral ischemia (10, 32, 33).

The Quantikine® QuicKit™ Human G-CSF Immunoassay is a one step, 80-minute solid phase ELISA designed to measure human G-CSF levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human G-CSF and antibodies raised against the recombinant protein. Results obtained using natural human G-CSF showed linear curves that were parallel to the standard curves obtained using the QuicKit™ standards. These results indicate that this kit can be used to determine relative mass values for natural human G-CSF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An anti-tag antibody has been pre-coated onto a microplate. Standards and samples are pipetted into the wells followed by an antibody cocktail. The antibody cocktail consists of an affinity tag labeled monoclonal capture antibody and an enzyme-linked polyclonal detection antibody, specific for human G-CSF. After washing away any unbound substances, a substrate solution is added to the wells and color develops in proportion to the amount of G-CSF bound. The color development is stopped and the intensity of the color is measured.

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 from other lots or sources.
- If samples generate values higher than the highest standard, dilute the samples with the calibrator diluent and repeat the assay.
- Any variation in 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® QuicKit™ 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.
- Ensure reagent addition to plate wells is uninterrupted.
- To ensure accurate results, proper adhesion of the plate sealer during the incubation step is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- 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. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate 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
QuicKit™ Coated Microplate	899063	96 well polystyrene microplate (12 strips of 8 wells) coated with an anti-tag monoclonal antibody.	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 G-CSF Standard	899150	2 vials of recombinant human G-CSF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human G-CSF Capture Ab Concentrate	899148	Lyophilized tagged monoclonal antibody specific for human G-CSF.	May be stored for up to 1 month at 2-8 °C.*
Human G-CSF Detection Ab Concentrate	899149	400 µL of a polyclonal antibody specific for human G-CSF conjugated to horseradish peroxidase with preservatives.	
Assay Diluent RD1X	895121	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-68	896030	21 mL of a buffered protein base 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	895032	6 mL of 2 N sulfuric 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
- Human G-CSF Controls (optional; R&D Systems®, Catalog # QC269)

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 - 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 or heparin 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: *Citrate plasma has not been validated for use in this assay.*
Samples with abnormally high levels of albumin interfere in this assay.

SAMPLE PREPARATION

Cell culture supernate samples may require dilution due to high endogenous levels.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human G-CSF Capture Ab Concentrate - Refer to the vial label for reconstitution volume. Reconstitute the Human G-CSF Capture Ab Concentrate with Assay Diluent RD1X. This reconstitution produces a 20X Capture Antibody stock. Allow the capture antibody to sit for a minimum of 5 minutes with gentle agitation prior to diluting. Once reconstituted, the 20X capture antibody stock can be stored for 4 weeks at 2-8 °C.

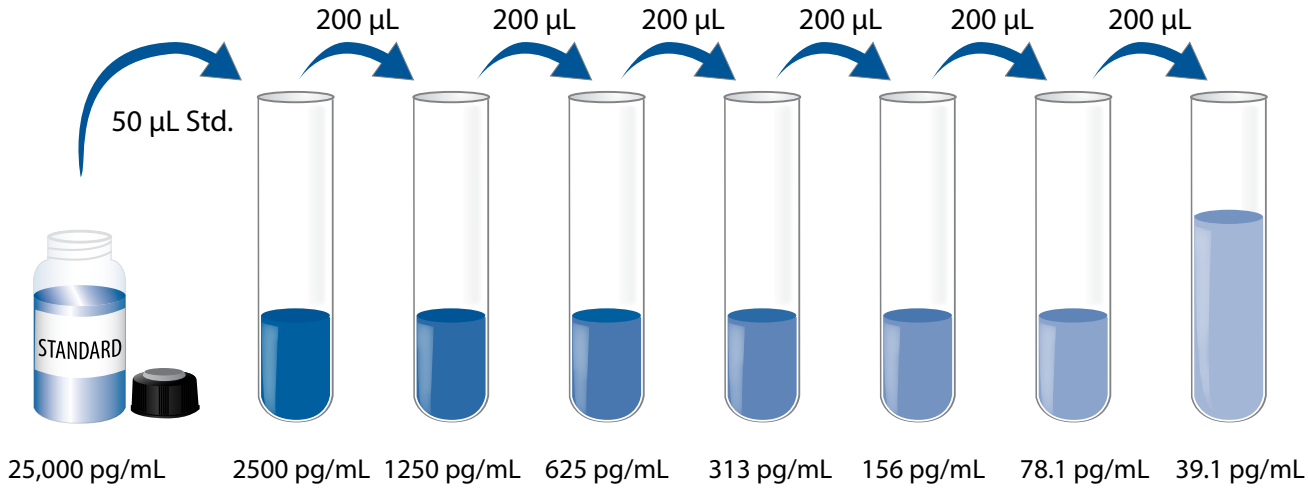
Antibody Cocktail - Dilute the reconstituted Capture Ab stock and the Detection Ab Concentrate 20-fold in Assay Diluent RD1X. For a full plate, add 300 µL of reconstituted Human G-CSF Capture Ab stock and 300 µL of Human G-CSF Detection Ab Concentrate to 5.4 mL of Assay Diluent RD1X.

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 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.

Human G-CSF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human G-CSF Standard with 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 450 µL of Calibrator Diluent RD5-68 into the 2500 pg/mL tube. Pipette 200 µL into the remaining tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 2500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-68 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, controls, and samples be assayed in duplicate.

1. Prepare all reagents, samples, and working standards 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 standard, control, or sample per well. A plate layout is provided to record standards and samples assayed.
4. Add 50 µL Antibody Cocktail to each well. Cover with the adhesive strip provided. Incubate for 1 hour at room temperature on a horizontal orbital microplate shaker (0.12″ orbit) set at 500 ± 50 rpm.
5. Aspirate each well and wash, repeating the process twice for a total of three 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 Substrate Solution to each well. Incubate for 20 minutes at room temperature **on the benchtop. Protect from light.**
7. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. 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

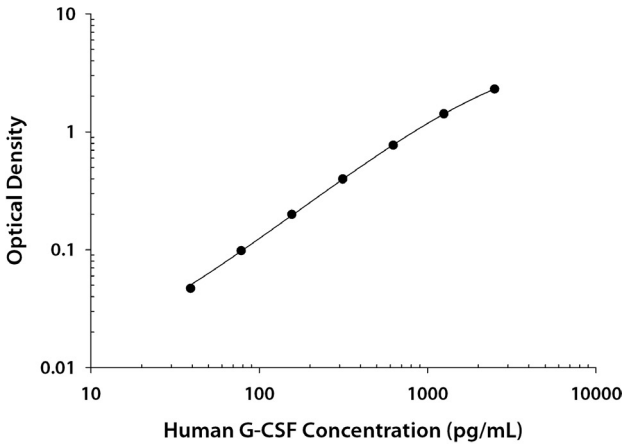
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 human G-CSF 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	
39.1	0.055		
	0.055	0.055	0.047
78.1	0.106		
	0.106	0.106	0.098
156	0.205		
	0.210	0.208	0.200
313	0.401		
	0.411	0.406	0.398
625	0.769		
	0.786	0.778	0.77
1250	1.411		
	1.446	1.429	1.421
2500	2.321		
	2.313	2.317	2.309

PRECISION

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

Inter-Assay Precision (Precision between assays)
Two samples of known concentration were tested in ten separate assays to assess inter-assay precision. Assays were performed by at least three technicians.

	Intra-Assay Precision		Inter-Assay Precision	
Sample	1	2	1	2
n	20	20	10	10
Mean (pg/mL)	260	1351	246	1426
Standard deviation	3.36	26.2	27.0	70.9
CV (%)	1.3	1.9	11.0	5.0

RECOVERY

The recovery of human G-CSF spiked to three levels in samples throughout the range of the assay was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	101	93-105%
Serum (n=2)	79	77-84%
EDTA plasma (n=2)	102	100-106%
Heparin plasma (n=2)	94	90-99%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human G-CSF were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=8)*	Serum (n=2)	EDTA plasma (n=2)	Heparin plasma (n=2)
1:2	Average % of Expected	102	97	99	91
	Range (%)	98-106	95-98	98-100	88-94
1:4	Average % of Expected	102	107	105	96
	Range (%)	87-107	106-108	104-107	91-100
1:8	Average % of Expected	104	124	111	105
	Range (%)	101-107	121-128	110-113	100-110
1:16	Average % of Expected	101	134	113	117
	Range (%)	93-109	134-135	112-113	113-120

*Samples were diluted prior to this assay.

SENSITIVITY

Eighteen assays were evaluated and the minimum detectable dose (MDD) of human G-CSF ranged from 0.676-3.57 pg/mL. The mean MDD was 1.59 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 human G-CSF produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Ten serum and plasma samples from apparently healthy volunteers were evaluated for the presence of human G-CSF in this assay. No medical histories were available for the donors used in this study. All samples measured less than the lowest human G-CSF standard, 39.1 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells (PBMCs) (1×10^6 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 ug/mL streptomycin sulfate. Cells were left unstimulated or stimulated with 10 ug/mL PHA for 1 or 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human G-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND
Stimulated	915	1184

ND=Non-detectable

THP-1 cells (2.5×10^5 cells/mL) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, 100 µg/mL streptomycin sulfate, and 50 µM β-mercaptoethanol. To differentiate the cells into macrophages, 50 ng/mL PMA was added when seeding cells and incubated overnight. The next day, media was removed, and cells were washed once with media before replacing normal media and incubated for 2 days. Cells were then left unstimulated or stimulated with 100 ng/mL or 1000 ng/mL LPS for 1 day. Aliquots of the cell culture supernates were removed and assayed for levels of human G-CSF.

Condition	Day 1 (pg/mL)
Unstimulated	ND
Stimulated with 100 ng/mL LPS	1813
Stimulated with 1000 ng/mL LPS	2721

ND=Non-Detectable

SPECIFICITY

This assay recognizes natural and recombinant human G-CSF.

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 low level recombinant human G-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

G-CSF R
GM-CSF
IFN-α/β R1
IL-3
IL-3 Rα
IL-9 R
M-CSF
M-CSF R

Recombinant mouse:

G-CSF
IL-3

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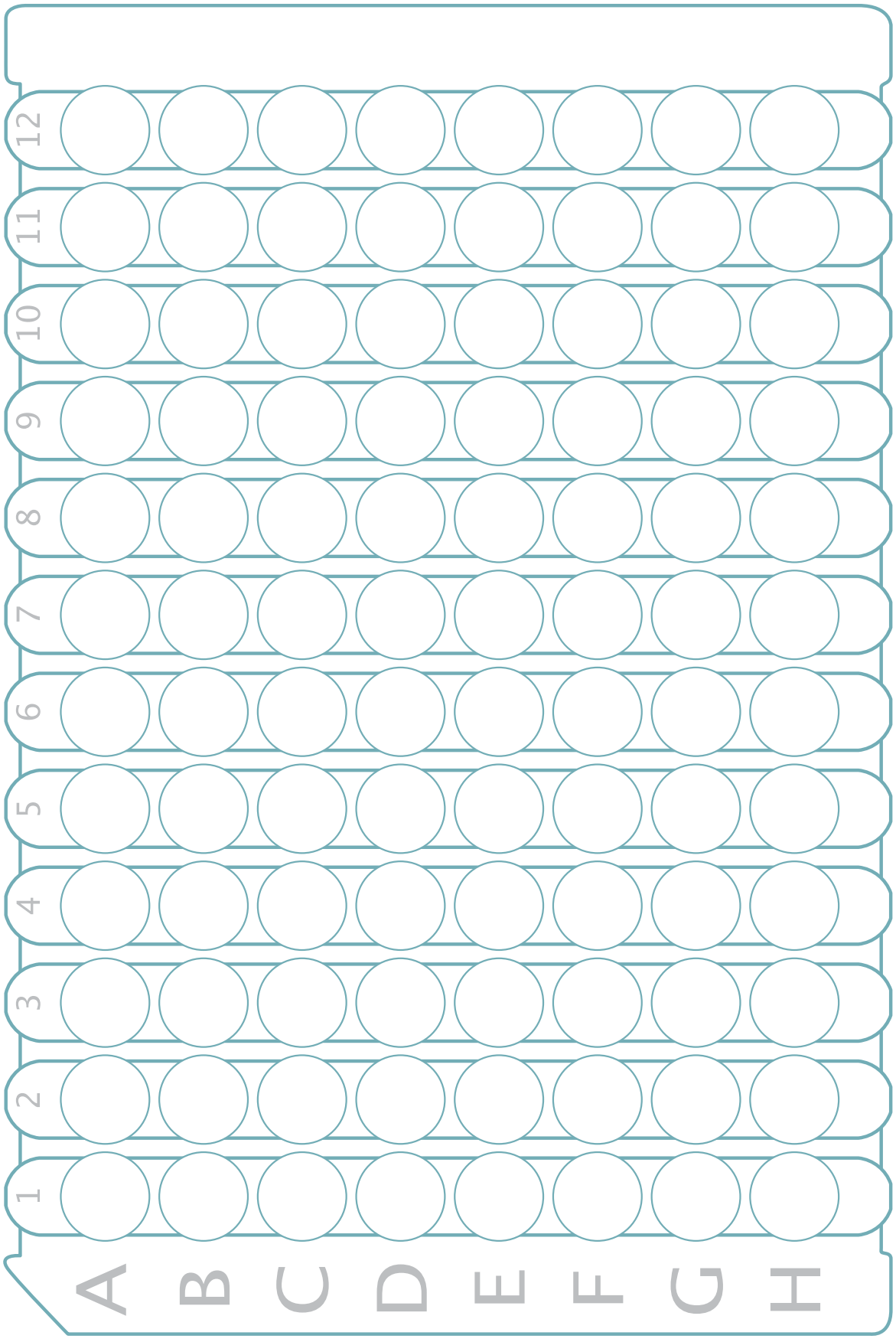
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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