Quantikine® HS ELISA

Human G-CSF Immunoassay

Catalog Number HSTCS0

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 HS Human G-CSF Immunoassay is a 4.5 hour solid phase ELISA designed to measure G-CSF in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human G-CSF and antibodies raised against the recombinant protein. It has been shown to accurately quantitate recombinant human G-CSF. Results obtained using natural human G-CSF showed linear curves that were parallel to the standard curves obtained using the Quantikine HS kit 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. A monoclonal antibody specific for human G-CSF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any G-CSF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human G-CSF is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of G-CSF bound in the initial step. 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 with those from other lots or sources.
- It is important that the Calibrator Diluent selected for the standard curve be consistent with the samples being assayed.
- If samples generate values higher than the highest standard, dilute the samples with the appropriate 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.
- 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.

<table>
<tr><th>PART</th><th>PART #</th><th>DESCRIPTION</th><th>STORAGE OF OPENED/
RECONSTITUTED MATERIAL</th></tr>
<tr><td>G-CSF HS Microplate</td><td>893687</td><td>96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against human G-CSF.</td><td>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.*</td></tr>
<tr><td>G-CSF HS Standard</td><td>893689</td><td>3000 pg of recombinant human G-CSF in a buffered protein base with preservatives; lyophilized.</td><td>Aliquot and store for up to 1 month at ≤ -20 °C* in a manual defrost freezer. Avoid multiple freeze-thaw cycles.</td></tr>
<tr><td>G-CSF HS Conjugate</td><td>893688</td><td>21 mL of a polyclonal antibody against human G-CSF conjugated to horseradish peroxidase with preservatives.</td><td rowspan="8">May be stored for up to 1 month at 2-8 °C.*</td></tr>
<tr><td>Assay Diluent RD1-60</td><td>895328</td><td>11 mL of a buffered protein base with preservatives.</td></tr>
<tr><td>Calibrator Diluent RD5-5</td><td>895485</td><td>21 mL of a buffered protein base with preservatives. For cell culture supernate samples.</td></tr>
<tr><td>Calibrator Diluent RD6P</td><td>895118</td><td>21 mL of buffered animal serum with preservatives. For serum/plasma samples. Use diluted 1:2 in this assay.</td></tr>
<tr><td>Wash Buffer Concentrate</td><td>895003</td><td>21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. May turn yellow over time.</td></tr>
<tr><td>Color Reagent A</td><td>895000</td><td>12 mL of stabilized hydrogen peroxide.</td></tr>
<tr><td>Color Reagent B</td><td>895001</td><td>12 mL of stabilized chromogen (tetramethylbenzidine).</td></tr>
<tr><td>Stop Solution</td><td>895032</td><td>6 mL of 2 N sulfuric acid.</td></tr>
<tr><td>Plate Sealers</td><td>N/A</td><td colspan="2">4 adhesive strips.</td></tr>
</table>

* 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; available from R&D Systems).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain ProClin® 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 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.

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REAGENT PREPARATION

Bring all reagents to room temperature before use.

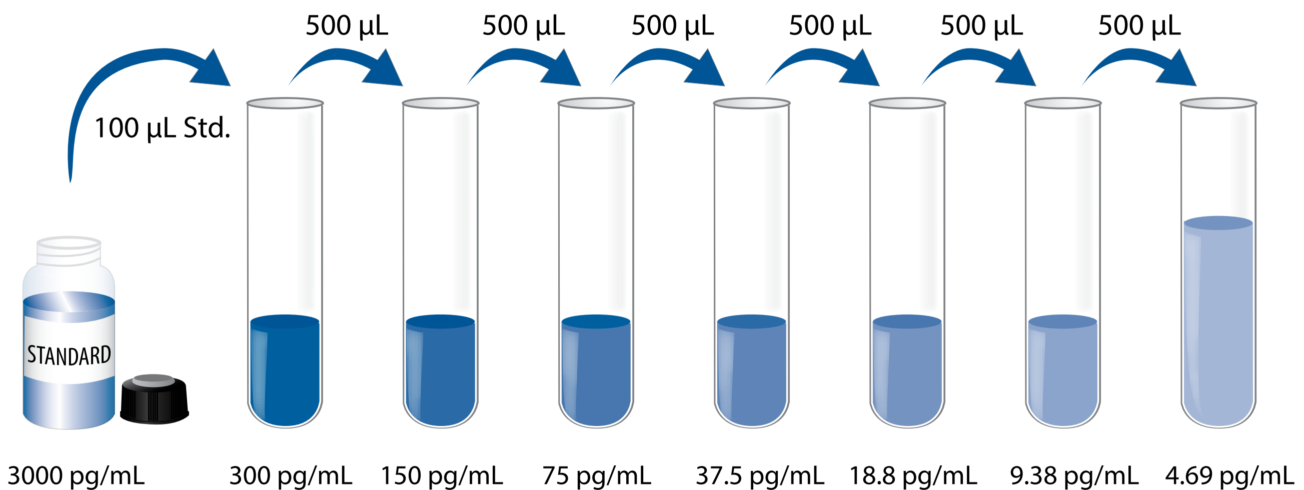
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD6P (diluted 1:2) - Add 10 mL of Calibrator Diluent RD6P to 10 mL of deionized or distilled water to prepare 20 mL of Calibrator Diluent RD6P (diluted 1:2).

G-CSF HS Standard - Reconstitute the G-CSF HS Standard with 1.0 mL deionized or distilled water. This reconstitution produces a stock solution of 3000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6P (diluted 1:2) (*for serum/plasma samples*) into the 300 pg/mL tube. Pipette 500 µL of the appropriate Calibrator Diluent into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 300 pg/mL standard serves as the high standard. The appropriate Calibrator Diluent 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 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 100 µL of Assay Diluent RD1-60 to each well.
4. Add 100 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 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 G-CSF HS Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. **Protect from light.**
   For Cell Culture Supernate samples: Incubate for 20 minutes at room temperature.
   For Serum/Plasma samples: Incubate for 30 minutes at room temperature.
9. 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.
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.).

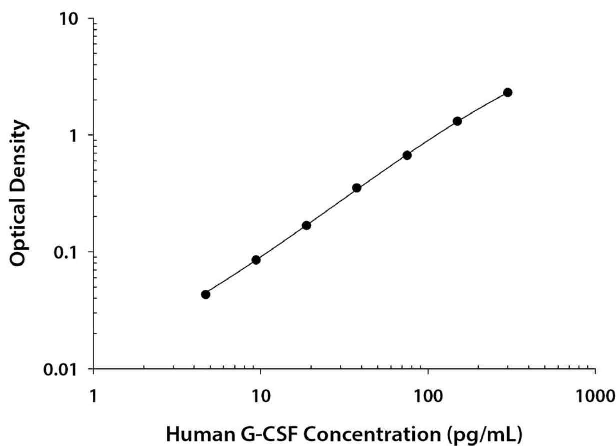
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the 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

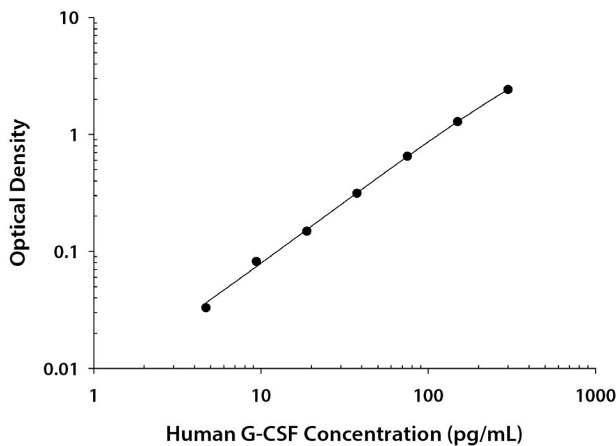
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CALIBRATOR DILUENT RD5-5



(pg/mL)	O.D.	Average	Corrected
0	0.046 0.047	0.047	—
4.69	0.089 0.090	0.090	0.043
9.38	0.132 0.132	0.132	0.085
18.8	0.214 0.216	0.215	0.168
37.5	0.396 0.402	0.399	0.352
75	0.716 0.716	0.716	0.669
150	1.326 1.383	1.355	1.308
300	2.212 2.485	2.349	2.302

CALIBRATOR DILUENT RD6P (diluted 1:2)



(pg/mL)	O.D.	Average	Corrected
0	0.067 0.072	0.070	—
4.69	0.098 0.100	0.103	0.033
9.38	0.137 0.141	0.152	0.082
18.8	0.217 0.220	0.219	0.149
37.5	0.378 0.390	0.384	0.314
75	0.718 0.722	0.720	0.650
150	1.341 1.366	1.354	1.284
300	2.447 2.530	2.489	2.419

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 forty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

CELL CULTURE SUPERNATE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	50.5	96.8	217	50.5	94.1	207
Standard deviation	2.1	3.2	9.7	4.0	7.0	10.5
CV (%)	4.2	3.3	4.5	7.9	7.4	5.1

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	60.0	113	243	62.6	117	260
Standard deviation	4.1	7.7	12.3	5.2	7.6	15.3
CV (%)	6.8	6.8	5.1	8.3	6.5	5.9

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	94-104%
Serum (n=4)	88	80-94%
EDTA plasma (n=4)	91	84-99%
Heparin plasma (n=4)	89	80-100%

SENSITIVITY

Seventy-two assays were evaluated and the minimum detectable dose (MDD) of G-CSF ranged from 0.34-4.62 pg/mL. The mean MDD was 1.71 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.

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of G-CSF in various matrices and 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)
1:2	Average % of Expected	103	103	102	103
	Range (%)	100-106	100-110	94-107	95-110
1:4	Average % of Expected	107	101	107	104
	Range (%)	104-112	92-111	93-118	94-110
1:8	Average % of Expected	105	104	100	103
	Range (%)	102-107	86-116	89-108	97-107
1:16	Average % of Expected	99	98	85	90
	Range (%)	91-115	78-118	68-108	71-112

CALIBRATION

This immunoassay is calibrated against a highly purified methionyl form of *E. coli*-expressed recombinant human G-CSF produced at R&D Systems.

The NIBSC/WHO 1st Internatonal Standard for Human G-CSF 88/502 was evaluated in this assay. The dose response curve of the 1st International Standard parallels the Quantikine HS standard curve. To convert sample values obtained with the Quantikine HS Human G-CSF kit to approximate NIBSC 88/502 values, use the equation below.

NIBSC (88/502) approximate value (IU/mL)= 0.050 x Quantikine HS Human G-CSF value (pg/mL).

SAMPLE VALUES

Serum/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.

Sample Type	Mean (pg/mL)	Range (pg/mL)	Standard Deviation (pg/mL)
Serum (n=35)	31.9	12.3-62.2	11.9
EDTA plasma (n=35)	31.1	13.8-68.0	11.9
Heparin plasma (n=35)	28.4	11.0-51.7	9.29

Cell Culture Supernates - Human peripheral blood leukocytes were cultured in DMEM supplemented with 5% fetal calf serum, 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 10 μg/mL PHA. Aliquots of the cell culture supernate were removed and assayed for levels of natural human G-CSF.

Condition	Day 1 (pg/mL)	Day 6 (pg/mL)
Unstimulated	ND	ND
Stimulated	4320	4400

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 mid-range rhG-CSF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
G-CSF R
GM-CSF
IL-3
M-CSF

Recombinant mouse:
IL-3
IL-3 Rα
IL-3 Rβ

Recombinant rat:
GM-CSF
IL-3

Other recombinants:
canine GM-CSF
feline GM-CSF
porcine GM-CSF

Recombinant mouse G-CSF cross-reacts approximately 0.1% in this assay.

For research use only. Not for use in diagnostic procedures.

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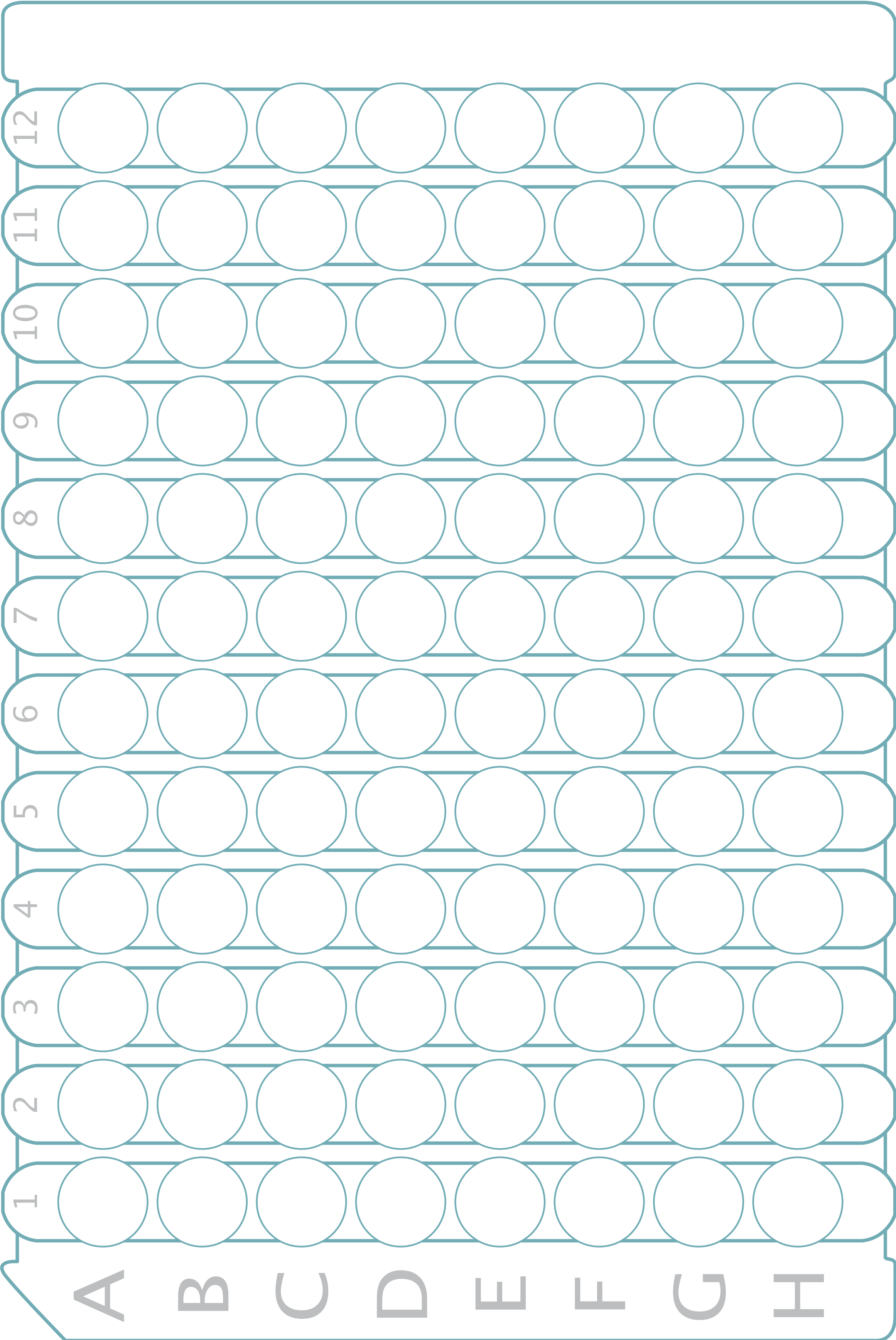
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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NOTES

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