Quantikine® ELISA

Human G-CSF Immunoassay

Catalog Number DCS50
SCS50
PDCS50

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 Human G-CSF Immunoassay is a 3.5-4.5 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 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.

PART	PART #	CATALOG # DCS50	CATALOG # SCS50	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human G-CSF Microplate	890247	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human G-CSF.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human G-CSF Standard	892152	2 vials	12 vials	Recombinant human G-CSF in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	May be stored for up to 2 weeks at 2-8 °C.*
Human G-CSF Conjugate	890270	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human G-CSF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6A	895013	1 vial	6 vials	21 mL/vial of buffered animal serum with preservatives. *For serum/plasma samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

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

DCS50 contains sufficient materials to run an ELISA on one 96 well plate.
SCS50 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDCS50). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Please refer to the literature accompanying your order for specific vial counts.

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.
- 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.

REAGENT PREPARATION

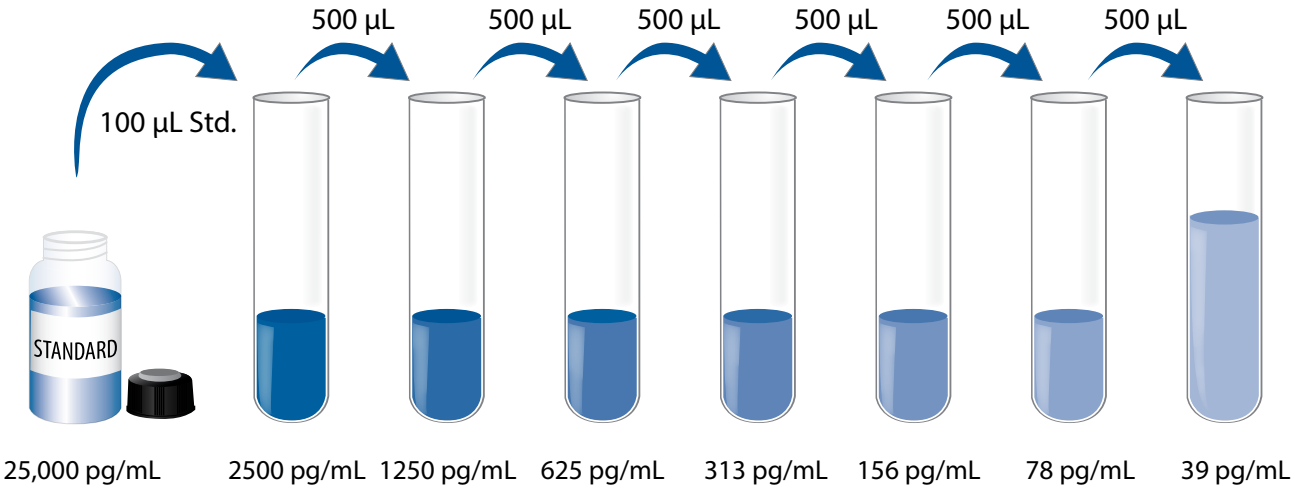
Bring all reagents to room temperature before use.

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.

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 900 µL of Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6A (*for serum/plasma samples*) into the 2500 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 2500 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 RD1W 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. A plate layout is provided to record the standards and samples assayed.
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 200 µL of Human G-CSF Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1 hour at room temperature.
   For Serum/Plasma Samples: Incubate for 2 hours at room temperature.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 20 minutes at room temperature. **Protect from light.**
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.).

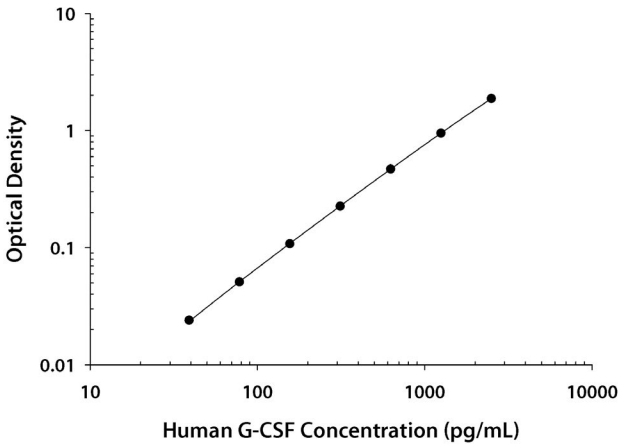
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

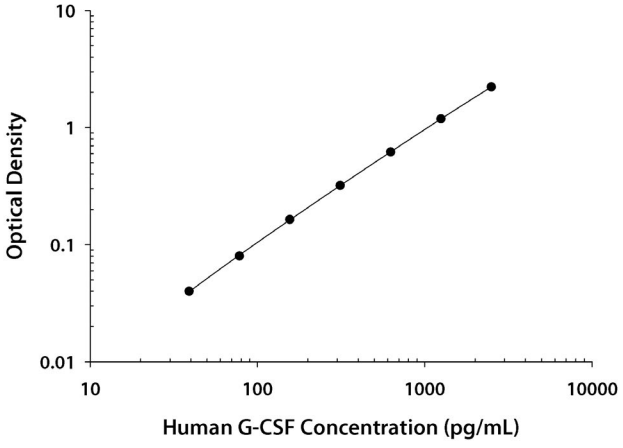
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.011 0.010	0.010	—
39	0.035 0.034	0.034	0.024
78	0.062 0.060	0.061	0.051
156	0.119 0.118	0.118	0.108
313	0.238 0.235	0.236	0.226
625	0.484 0.477	0.480	0.470
1250	0.968 0.953	0.960	0.950
2500	1.886 1.877	1.882	1.872

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.014 0.013	0.014	—
39	0.055 0.053	0.054	0.040
78	0.096 0.093	0.094	0.080
156	0.180 0.176	0.178	0.164
313	0.333 0.335	0.334	0.320
625	0.638 0.628	0.633	0.619
1250	1.220 1.179	1.200	1.186
2500	2.257 2.209	2.233	2.219

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 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	20	20	20
Mean (pg/mL)	177	515	1047	143	818	1514
Standard deviation	4.5	9.4	22.0	17.0	72.0	77.1
CV (%)	2.5	1.8	2.1	11.9	8.8	5.1

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	280	827	1696	176	1094	2169
Standard deviation	7.8	14.1	19.5	7.3	34.6	81.6
CV (%)	2.8	1.7	1.1	4.1	3.2	3.8

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	94	85-110%
Serum (n=5)	88	75-106%
EDTA plasma (n=5)	93	80-117%
Heparin plasma (n=5)	87	76-103%
Citrate plasma (n=5)	90	78-111%

LINEARITY

To assess the linearity of the assay, samples were spiked with high concentrations of human 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=5)	Serum (n=5)	EDTA plasma (n=5)	Heparin plasma (n=5)	Citrate plasma (n=5)
1:2	Average % of Expected	103	115	106	111	110
	Range (%)	99-109	112-121	105-108	109-113	105-121
1:4	Average % of Expected	105	122	113	118	118
	Range (%)	100-110	117-130	109-115	113-121	115-128
1:8	Average % of Expected	108	133	110	126	110
	Range (%)	103-114	125-143	97-118	122-130	76-127
1:16	Average % of Expected	———	146	105	130	132
	Range (%)	———	138-157	95-121	109-140	109-144

SENSITIVITY

The minimum detectable dose (MDD) of human G-CSF is typically less than 20 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 methionyl form of *E. coli*-expressed recombinant human G-CSF containing 175 amino acid residues. The NIBSC/WHO 1st International Standard for G-CSF 88/502 was evaluated in this kit. The dose response curve of the interim reference material parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine kit to approximate NIBSC (88/502) International units, use the equation below.

NIBSC (88/502) approximate value (IU/mL)= 0.12 x Quantikine Human G-CSF value (pg/mL).

SAMPLE VALUES

Serum/Plasma - Thirty-seven serum and plasma samples from apparently healthy volunteers were evaluated for the presence of 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 pg/mL.

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% 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 culture supernate were removed on days 1 and 5 and assayed for levels of natural human G-CSF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	258	118
Stimulated	854	1139

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:

GM-CSF
IL-3
IL-3 Rβ
M-CSF

Recombinant mouse:

G-CSF
IL-3

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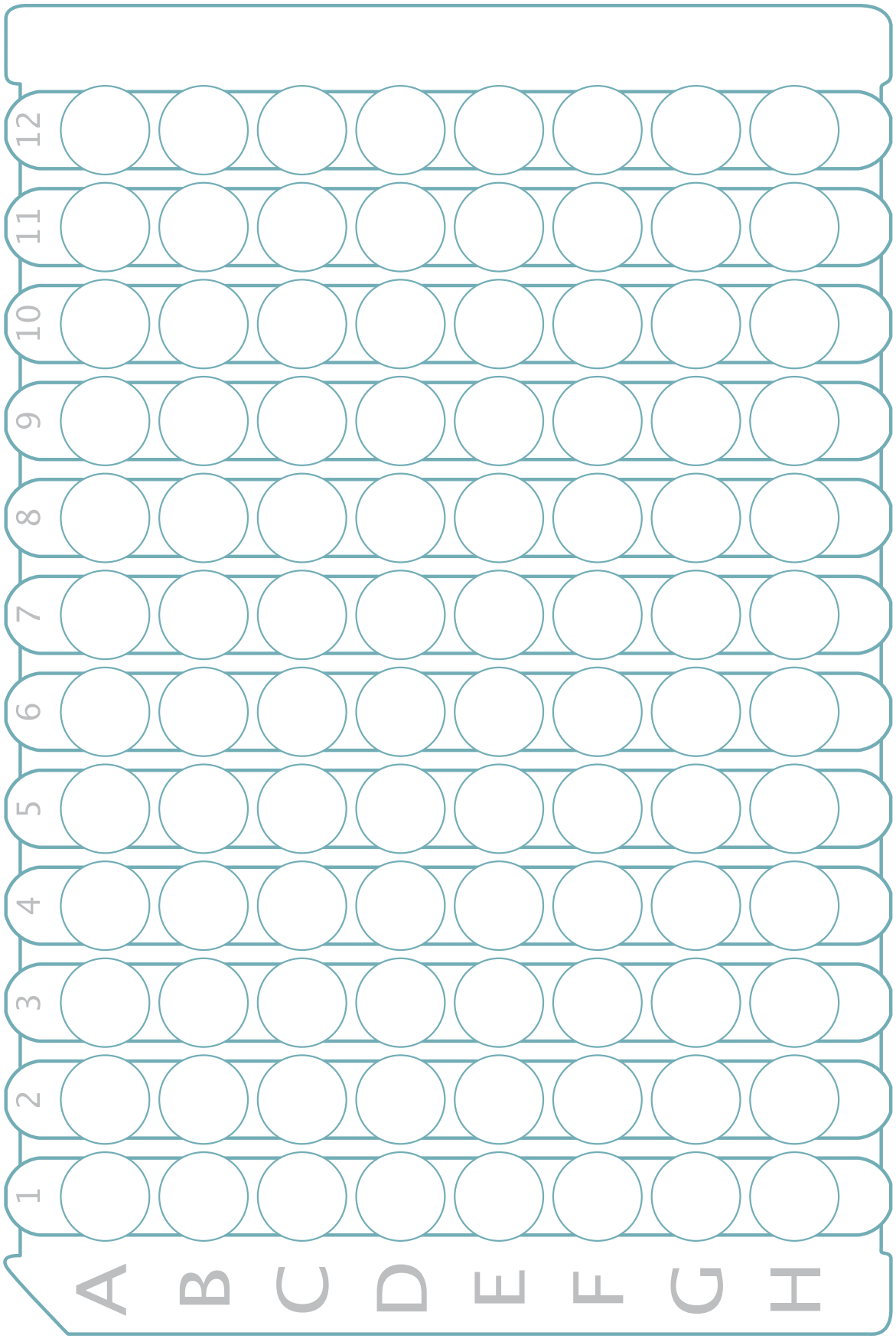
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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