Quantikine® ELISA

Human HGF Immunoassay

Catalog Number DHG00
SHG00
PDHG00

For the quantitative determination of human Hepatocyte Growth Factor (HGF) 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

Hepatocyte Growth Factor (HGF), also known as scatter factor, hepatopoietin A, and mammary growth factor, is a pleiotropic glycoprotein that regulates the growth and migration of diverse cell types. It is structurally similar to the S1 peptidase Plasminogen. HGF contains an N-terminal PAN/APPLE-like domain, four Kringle domains, and a catalytically inactive serine proteinase-like domain (1, 2). Alternative splicing generates human HGF isoforms that lack the proteinase-like domain and different numbers of the Kringle domains. HGF is secreted as an inactive single chain propeptide that can circulate as a soluble molecule or associate with the extracellular matrix (3, 4). At sites of tissue damage, the propeptide is cleaved after the fourth Kringle domain by serine proteases including HGF Activator and uPA (4-8). The resulting bioactive HGF consists of a disulfide-linked heterodimer of a 60 kDa N-terminal alpha chain and a 30 kDa C-terminal beta chain (4, 5, 9). The serum levels of HGF are elevated in a wide range of pathologies including liver damage (10, 11), acute kidney failure (12), myocardial infarction (13), type 1 diabetes (14), obesity (15), and cancer (16-23), as well as in the synovial fluid of rheumatoid arthritis patients (24). Human HGF shares 91%-94% amino acid sequence identity with bovine, canine, feline, mouse, and rat HGF. HGF demonstrates marked species cross-reactivity (25).

HGF exerts its biological activity through the widely expressed receptor tyrosine kinase, HGF R/c-MET (26, 27). This receptor undergoes N-linked glycosylation followed by proteolytic cleavage into 50 kDa N-terminal alpha and 145 kDa C-terminal beta chains (28). The strictly extracellular alpha chain remains disulfide-linked to the beta chain which contains the remaining extracellular, transmembrane, and cytoplasmic domains (26, 27). HGF also binds heparan sulfate proteoglycans, and these interactions enhance the ability of HGF to bind and activate HGF R (29, 30). In the absence of ligand, HGF R forms noncovalent complexes with a variety of membrane proteins including CD44v6, CD151, EGF R, Fas, Integrin α6/β4, Plexins B1, B2, B3, and MSP R/Ron (31-38). Ligation of one complex component can trigger activation of the other, followed by cooperative signaling effects (31-38). Formation of some of these heteromeric complexes is a requirement for epithelial cell morphogenesis and tumor cell invasion (34-36). Overexpression and the production of alternate forms of HGF R are implicated in the development of many human cancers (39).

HGF is expressed by fibroblasts, adipocytes, smooth muscle cells, and endothelial cells (1). Expression of HGF R, on the other hand, is found mainly on epithelial cells, suggesting that HGF acts in a paracrine fashion to mediate interactions between stromal and epithelial cells (40). HGF induces the proliferation and migration of epithelial cells as well as multiple other cell types including hepatocytes, chondrocytes, keratinocytes, melanocytes, and endothelial cells (1). It is mitogenic toward most tumor cells but can conversely inhibit their proliferation in some cases (39, 41, 42). During organogenesis, tissue repair, and angiogenesis, HGF promotes epithelial/endothelial morphogenesis by inducing cell scattering and branching tubulogenesis (1, 25, 43, 44). The ability of HGF to regulate angiogenesis and the motility of epithelial cells underlie its importance in the development of solid tumors (39). In addition to its morphogenetic effects, HGF induces a range of responses in diverse tissues (1). It supports the survival, proliferation, and insulin productivity of pancreatic islet cells (45). It functions as a neurotrophic factor during development and in the response to injury (46, 47). It also suppresses inflammation by inducing dendritic cell tolerization, Treg induction, and Th2 bias while inhibiting T cell activation, IL-17 expression, and inflammatory cell infiltration (48-50).

INTRODUCTION *CONTINUED*

The Quantikine Human HGF Immunoassay is a 4.25-4.5 hour solid phase ELISA that is designed to measure HGF levels in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human pro-HGF and antibodies raised against the recombinant factor, and has been shown to quantitate recombinant human HGF accurately. Results obtained using natural HGF 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 HGF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human HGF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any HGF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human HGF 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 HGF 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.
- 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.

PRECAUTIONS

HGF is detectable in saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.

Calibrator Diluent RD6X contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	CATALOG # DHG00	CATALOG # SHG00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human HGF Microplate	890235	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human HGF.	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 HGF Standard	890237	1 vial	6 vials	Recombinant human pro-HGF in a buffered protein base with preservatives; lyophilized. *Refer to vial label for reconstitution volume.*	Aliquot and store for up to 1 month at ≤ -20 °C in a manual defrost freezer.* Avoid repeated freeze-thaw cycles.
Human HGF Conjugate	890236	1 vial	6 vials	21 mL/vial of polyclonal antibody specific for human HGF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1W	895117	2 vials	12 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	1 vial	6 vials	21 mL/vial of a concentrated buffered protein base with preservatives. *For cell culture supernate samples. Use diluted 1:5.*	
Calibrator Diluent RD6X	895152	1 vial	6 vials	21 mL/vial of 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 preservatives. *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.

DHG00 contains sufficient materials to run an ELISA on one 96 well plate.
SHG00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems, Catalog # PDHG00). 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.
- 100 mL and 500 mL graduated cylinders.
- **Polypropylene** test tubes for dilution of standards.
- Human HGF Controls (R&D Systems, Catalog # QC21).

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 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: *Heparin plasma is not recommended 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 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

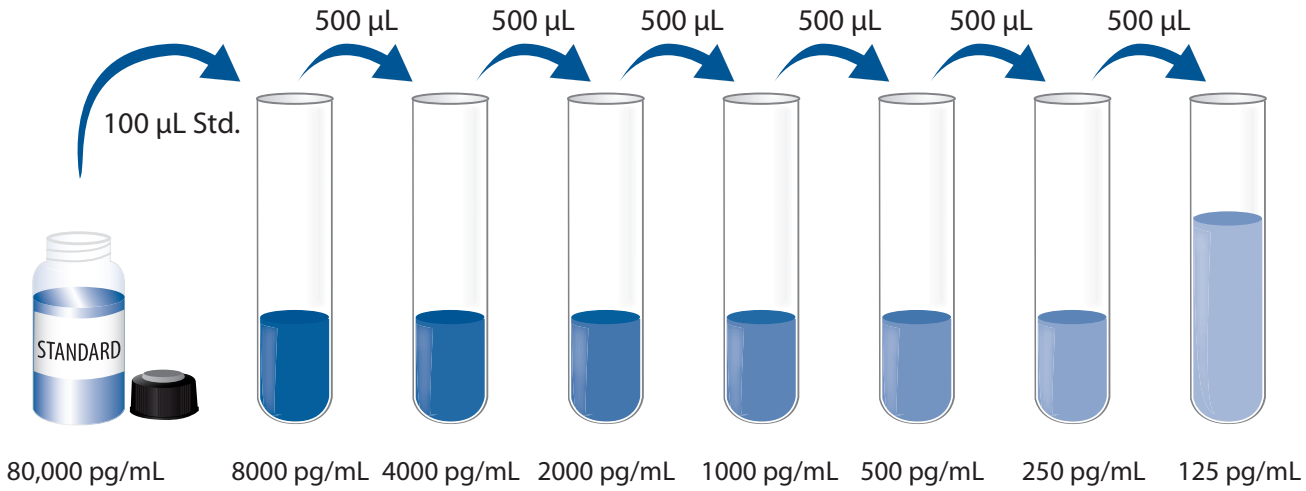
Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Calibrator Diluent RD5P (diluted 1:5) - Add 20 mL of Calibrator Diluent RD5P Concentrate to 80 mL of deionized or distilled water to prepare 100 mL of Calibrator Diluent RD5P (diluted 1:5).

Human HGF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human HGF Standard with deionized or distilled water. This reconstitution produces a stock solution of 80,000 pg/mL. Mix the standard by inversion or brief vortex for 5-10 seconds to ensure complete reconstitution and then allow the standard to sit for a minimum of 15 minutes prior to making dilutions.

Note: *Do not use rocker or extended vortex.*

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:5) (*for cell culture supernate samples*) or Calibrator Diluent RD6X (*for serum/plasma samples*) into the 8000 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 8000 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.

Note: *HGF is detectable in saliva. Take precautionary measures to prevent contamination of the kit reagents while running the assay.*

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 150 µL of Assay Diluent RD1W per well.
4. Add 50 µL of Standard, control, or sample per well. Ensure reagent addition is uninterrupted and completed **within 15 minutes.** Mix by gently tapping the plate. 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 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 HGF Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1.75 hours 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 30 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 if 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.).

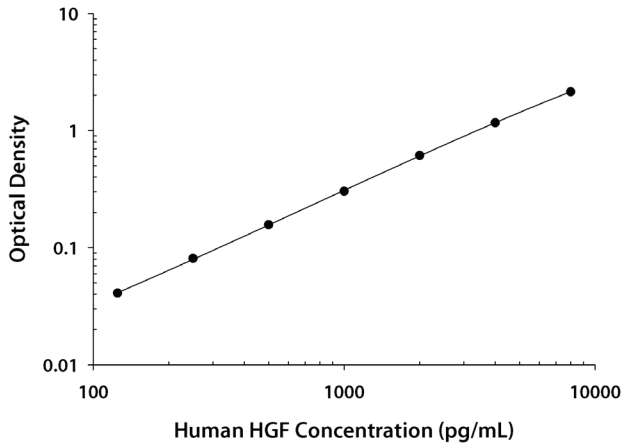
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best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

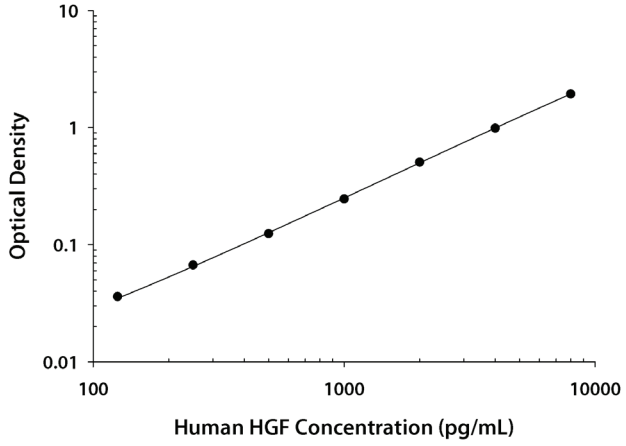
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.046 0.048	0.047	—
125	0.089 0.087	0.088	0.041
250	0.128 0.127	0.128	0.081
500	0.206 0.203	0.204	0.157
1000	0.366 0.333	0.350	0.303
2000	0.649 0.672	0.660	0.613
4000	1.199 1.226	1.212	1.165
8000	2.216 2.167	2.192	2.145

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.055 0.057	0.056	—
125	0.091 0.094	0.092	0.036
250	0.125 0.121	0.123	0.067
500	0.182 0.178	0.180	0.124
1000	0.311 0.294	0.302	0.246
2000	0.580 0.543	0.562	0.506
4000	1.067 1.020	1.044	0.988
8000	2.074 1.908	1.991	1.935

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)	425	991	3869	409	1058	4058
Standard deviation	30.2	43.2	234	29.4	75.1	264
CV (%)	7.1	4.4	6.0	7.2	7.1	6.5

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)	339	999	3759	363	967	3790
Standard deviation	23.7	56.2	154	25.8	81.2	205
CV (%)	7.0	5.6	4.1	7.1	8.4	5.4

RECOVERY

The recovery of human HGF spiked to three different levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	92	86-101%
Serum (n=5)	99	91-108%
EDTA plasma (n=5)	100	85-111%
Citrate plasma (n=5	105	99-114%

SENSITIVITY

The minimum detectable dose (MDD) of human HGF is typically less than 40 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 human HGF 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)	Citrate plasma (n=5)
1:2	Average % of Expected	104	100	100	102
	Range (%)	95-107	98-101	97-104	98-106
1:4	Average % of Expected	104	97	98	103
	Range (%)	98-107	96-99	93-107	97-111
1:8	Average % of Expected	106	96	97	101
	Range (%)	102-108	93-100	89-109	97-107
1:16	Average % of Expected	104	96	98	102
	Range (%)	94-109	92-106	86-110	93-113

CALIBRATION

This immunoassay is calibrated against a highly purified *Sf* 21-expressed recombinant human pro-HGF produced at R&D Systems.

To convert sample values obtained with the Quantikine Human HGF kit to approximate NIBSC 96/556 International Units, use the equation below.

NIBSC (96/556) approximate value (IU/mL) = 0.0003 x Quantikine Human HGF value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human HGF 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=60)	1257	671-1992	289
EDTA plasma (n=60)	787	469-1113	136
Citrate plasma (n=71)	440	251-742	102

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 units/mL penicillin, and 100 µg/mL streptomycin sulfate. The cells were cultured unstimulated or stimulated with 10 µg/mL PHA for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human HGF.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	548
Stimulated	152	145

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human HGF.

The factors listed below were prepared at 50 ng/mL in each Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range rhHGF control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:
EGF
G-CSF
GM-CSF
HGF R
M-CSF
PDGF-AA
PDGF-AB
PDGF-BB
TGF-β1
VEGF

Recombinant mouse:
HGF

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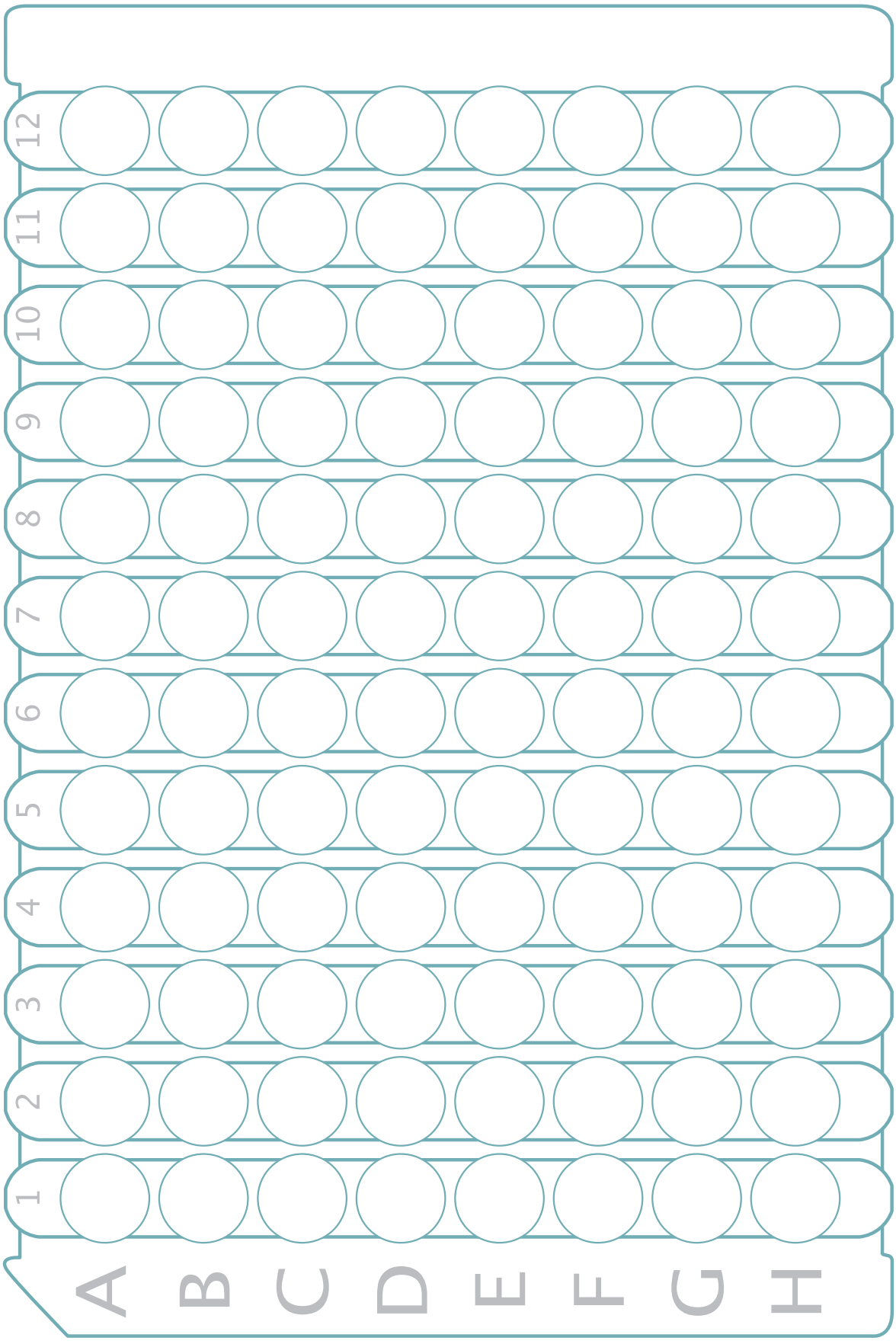
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES