Quantikine® ELISA

Human KGF/FGF-7 Immunoassay

Catalog Number DKG00

For the quantitative determination of human Keratinocyte Growth Factor (KGF) 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

Human Keratinocyte Growth Factor (KGF) is a single chain, heparin-binding, 28 kDa glycoprotein that was originally isolated from media conditioned by the growth of human embryonic lung fibroblasts (1, 2). Also known as FGF-7 (or fibroblast growth factor 7), it is part of the rapidly-expanding fibroblast growth factor family that currently includes 14 members (3-6). Mature KGF is 163 amino acid (aa) residues in length, and contains five cysteines, which are not necessary for mitogenic activity, but do contribute to heparin binding (2, 7). Within the FGF family, KGF shows 29% aa sequence identity to FGF-2 and 38% aa sequence identity to FGF-3 (8). Cells reported to express KGF are fibroblasts (1, 9), embryonic mesenchymal cells (10-12), and smooth muscle cells (13).

The receptor for KGF (KGF R) is a restricted-expression splice variant of the bek (bacterially-expressed kinase) gene product, a cell surface receptor with tyrosine kinase activity, also designated FGF R2 (FGF Receptor 2) (14-16). FGF R2 as a full-length, unspliced (or standard), 135 kDa, type I (extracelluar N-terminus) transmembrane glycoprotein with an extracellular domain containing three Ig-like domains plus a heparin-binding motif in the interdomain sequence that connects the N-terminal (D1) and middle (D2) Ig-domains (16). This standard receptor form is expressed ubiquitously in connective tissue cells (17) and binds FGF-1, FGF-2, and FGF-4 with high affinity (Kd ~ 100 pM). FGF-5 and FGF-9 also bind, but with lower affinity (Kd ~2 nM) (16, 18-20). The KGF R splice variant differs from the standard receptor only within a 49 aa residue sequence found in the third (or membrane proximal) Ig-like domain (D3) (15, 21). Although this change has little effect on FGF-1 binding (Kd = 600 pM), its presence decreases FGF-2 binding (Kd = 3 nM) (15) and allows for KGF binding (Kd = 200 pM). As with KGF, the number of cells expressing KGF R are few and limited to epithelial cell types such as keratinocytes (22), transitional epithelium (but not umbrella cells) (23), gastric columnar epithelial cells (24), embryonic lung epithelium (10), mammary epithelium (25), and hepatocytes (26). In addition to the KGF R, KGF also binds to heparan sulfate proteoglycans (HSPG). In general, the role that HSPGs play in the mediation of the biological activities of FGFs is unclear, although they are thought to facilitate binding of FGFs to their high-affinity tyrosine kinase receptors. It has been suggested that HSPGs may hold two FGF molecules in close proximity, thus allowing two individual FGF-FGF R complexes to dimerize or, alternatively, form one FGF-HSPG complex that can actively bind two separate FGF receptors (16). For KGF in particular, however, the HSPGs have been found to have either no effect or an inhibitory effect on KGF activity. Thus no concensus exists concerning the importance of heparan sulfate for KGF binding and biological activity (27).

Functionally, KGF has been suggested to be a paracrine effector for a number of different epithelial cell types (1, 10, 11, 26, 27). Synthesized by dermal or lamina propria fibroblasts, it is proposed to act locally on the overlying epithelial sheet. In addition to its ability to induce cell proliferation (27), it may also promote epithelial differentiation (12). During wound healing, KGF's role as a re-epithelializing agent is complemented by the presence of proinflammatory molecules which appear as a result of tissue damage. Cytokines such as IL-1(α and β) and IL-6 not only activate local connective tissue cells, resulting in foreign body clearance and tissue remodeling, but also stimulate the production of fibroblast KGF which contributes to re-epithelialization and wound closure (9, 28, 29).

The Quantikine Human KGF/FGF-7 Immunoassay is a 5.25-5.5 hour solid phase ELISA designed to measure KGF in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human KGF and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate the recombinant KGF accurately. Results obtained measuring natural human KGF showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine kit standards. These results indicate that the Quantikine kit can be used to determine relative mass values for natural human KGF.

PRINCIPLE OF THE ASSAY

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

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 KGF Microplate	890536	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human KGF.	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 KGF Standard	890538	2 vials of recombinant human KGF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Discard after use. Use a fresh standard for each assay.
Human KGF Conjugate	890537	21 mL of a polyclonal antibody specific for human KGF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-25	895229	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5R	895190	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-15	895244	21 mL of buffered protein base with preservatives. *For serum/plasma samples.*	
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.
- **Polypropylene** test tubes for dilution of standards.
- Human KGF Controls (optional; R&D Systems, Catalog # QC21).

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. 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

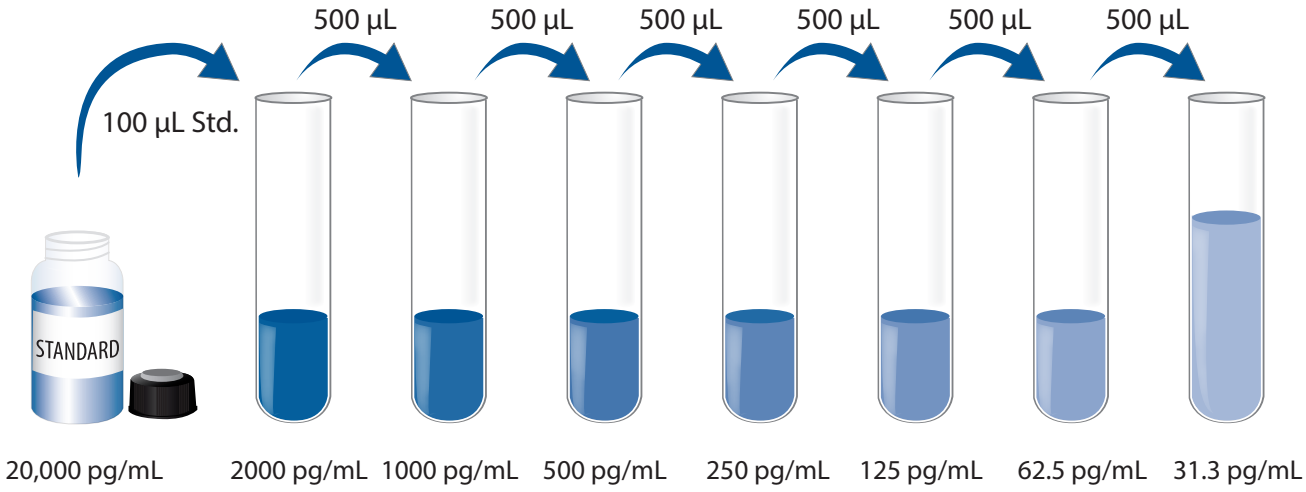
Bring all reagents to room temperature before use.

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 KGF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human KGF Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Use polypropylene tubes. Pipette 900 µL of the Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-15 (*for serum/plasma samples*) into the 2000 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 2000 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, 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 RD1-25 to each well.
4. Add 100 µL of Standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 3 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 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 KGF 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. If color change does not appear uniform, gently tap the plate to ensure thorough mixing. 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.).

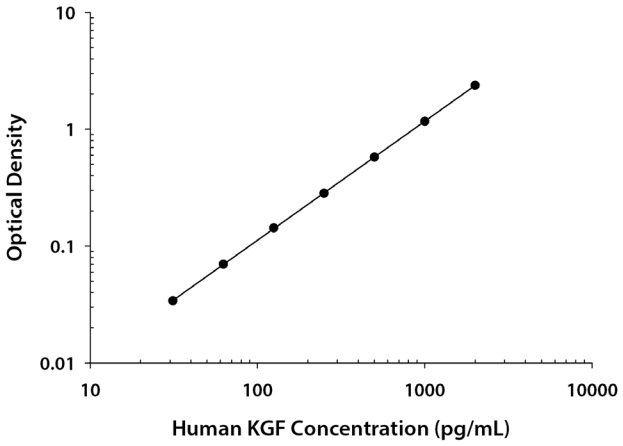
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human KGF concentrations versus the log of the O.D. on a linear scale, and the best fit line can be determined by regression analysis.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

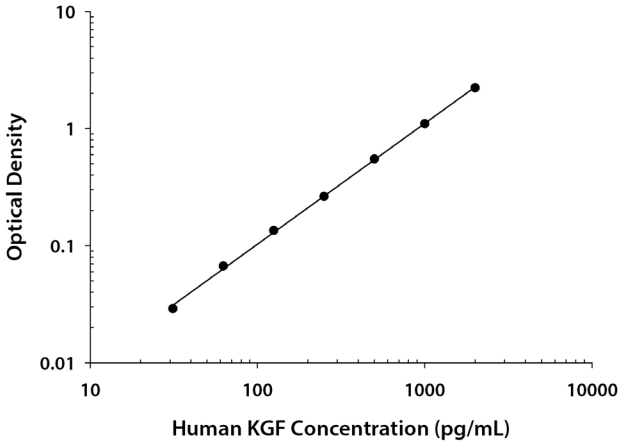
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.026 0.033	0.030	—
31.3	0.066 0.063	0.064	0.034
62.5	0.100 0.100	0.100	0.070
125	0.174 0.172	0.173	0.143
250	0.319 0.307	0.313	0.283
500	0.608 0.607	0.608	0.578
1000	1.157 1.235	1.196	1.166
2000	2.466 2.342	2.404	2.374

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.037 0.041	0.039	—
31.3	0.069 0.066	0.068	0.029
62.5	0.108 0.103	0.106	0.067
125	0.177 0.172	0.174	0.135
250	0.301 0.302	0.302	0.263
500	0.594 0.584	0.589	0.550
1000	1.159 1.119	1.139	1.100
2000	2.267 2.256	2.262	2.223

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)	82.4	248	1031	86.5	266	1108
Standard deviation	3.2	8.8	55.2	4.8	11.4	62.0
CV (%)	3.9	3.5	5.4	5.5	4.3	5.6

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)	97.9	298	1229	95.5	294	1221
Standard deviation	3.3	8.8	42.8	7.4	15.6	63.5
CV (%)	3.4	3.0	3.5	7.7	5.3	5.2

RECOVERY

The recovery of human KGF 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)	102	95-105%
Serum (n=5)	93	85-100%
EDTA plasma (n=5)	96	85-104%
Heparin plasma (n=5)	90	85- 99%
Citrate plasma (n=5)	91	86-98%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human KGF were serially 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	99	100	101	103	104
	Range (%)	98-100	98-102	99-104	101-106	103-107
1:4	Average % of Expected	99	103	103	104	104
	Range (%)	97-102	101-107	102-106	101-106	100-108
1:8	Average % of Expected	96	105	104	106	108
	Range (%)	93-99	102-108	102-106	104-108	104-115
1:16	Average % of Expected	94	103	103	107	103
	Range (%)	89-97	98-106	101-107	104-116	96-108

SENSITIVITY

The minimum detectable dose (MDD) of human KGF is typically less than 15 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 *E. coli*-expressed recombinant human KGF produced at R&D Systems.

The NIBSC/WHO Reference Reagent 03/148 for KGF was evaluated in this kit. The dose response curve of the reference reagent 03/148 parallels the Quantikine standard curve. To convert sample values obtained with the Quantikine Human KGF kit to approximate NIBSC/WHO 03/148 units, use the equation below.

NIBSC (03/148) value (U/mL) = 0.0014 x Quantikine Human KGF value (pg/mL).

SAMPLE VALUES

Serum/Plasma - Eighty-five serum and plasma samples from apparently healthy volunteers were evaluated for the presence of KGF in this assay. All samples measured below the lowest Human KGF Standard, 31.3 pg/mL. No medical histories were available for the donors used in this study.

Cell Culture Supernates - NHDF human normal dermal fibroblasts were cultured in fibroblast basal media supplemented with 1 µg/mL of recombinant human FGF, 5 mg/mL insulin, 50 mg/mL gentamycin, 50 µg/mL amphotericin-B, and 10% heat inactivated fetal calf serum. Cells were stimulated with the agents listed in the table below. Aliquots of the cell culture supernates were removed after 2 days and assayed for levels of natural human KGF.

Stimulant	Day 2 (pg/mL)
Control	106
human IL-1β	258
human TNF-α	208
human IL-6	110

SPECIFICITY

This assay recognizes natural and recombinant human KGF.

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 rhKGF control were also assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
Epo
FGF-4
FGF-5
FGF-6
FGF acidic
FGF basic
HB-EGF
HGF
IL-1α
IL-1β
IL-6
LAP (TGF-β1)
LIF
PD-ECGF
PDGF-AA
PDGF-AB
PDGF-BB
VEGF

Recombinant mouse:

IL-1α
IL-1β
IL-6
LIF

Recombinant amphibian:

TGF-β5

Natural proteins:

bovine FGF acidic
bovine FGF basic
human PDGF
porcine PDGF
human TGF-β1
porcine TGF-β1
porcine TGF-β2

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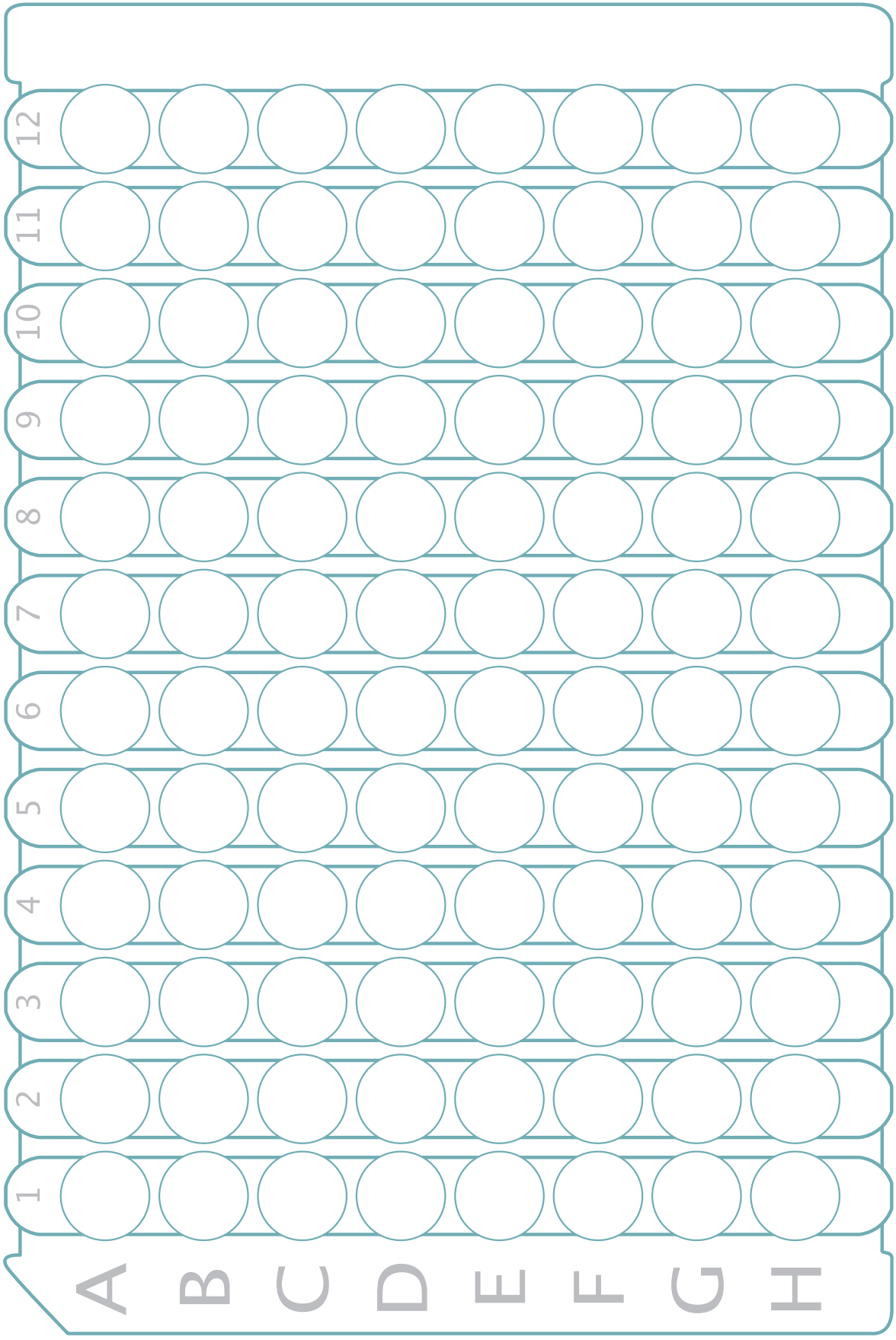
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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