Quantikine® ELISA

Human Growth Hormone Immunoassay

Catalog Number DGH00
SGH00
PDGH00

For the quantitative determination of human Growth Hormone concentrations in cell culture supernates, serum, and plasma.

Note: The standard reconstitution method has changed. Please read this package insert in its entirety before using this product.

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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614 McKinley Place NE, Minneapolis, MN 55413, USA
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19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
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24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
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INTRODUCTION

Human Growth Hormone (hGH), also known as Somatotropin, is a secreted protein best known for its role in the regulation of somatic growth and metabolism. It is encoded by the normal GH (GH-N/GH1) gene on human chromosome 17 in a cluster that also includes the genes for variant GH (GH-V/GH2), Chorionic Somatomammatotropin Gene A (CS-A), CS-B, and CS-like gene (1). The hGH cDNA encodes a 217 amino acid (aa) residue precursor protein with a putative 26 aa signal peptide (2-4). The gene products encoded by the GH-N and GH-V genes are highly homologous, differing by 13 amino acids spaced throughout the proteins (5). In contrast to GH-N, which is highly expressed by the somatotropic cells of the anterior pituitary, GH-V is expressed in the syncyciotrophoblasts of the placenta and circulating levels increase progressively during pregnancy (6, 7). hGH is molecularly heterogeneous with various forms existing through alternative splicing, proteolytic fragmentation, post-translational modification, and association with binding proteins (8). For instance, hGH may exist in at least 4 different alternatively spliced isoforms (9-11). The two most studied hGH forms include the full-length mature protein with a molecular weight of 22 kDa, and a 20 kDa gene product differing by the deletion of aa residues 32-46 (11, 12). It has been estimated that the 22 kDa monomer form accounts for approximately half of the hGH found in circulation, while the 20 kDa form makes up 5-10% of the total amount of hGH (8, 10, 13, 14). The remaining hGH consists primarily of oligomeric forms and other fragments (5, 8, 14, 15).

hGH is released into the circulation in pulsatile fashion and serum concentrations are generally higher in females than males (16-18). GH secretion is regulated by the activities of several peptides and proteins in a manner that may vary between species (19). GH-releasing Hormone and the peptide hormone Ghrelin enhance hGH secretion, while Somatostatin suppresses secretion (20, 21). Other molecules that may regulate hGH levels include Thyrotropin-releasing Hormone, Leptin, Galanin, Neuropeptide Y, and Cortisol (19, 22-24).

hGH is a pleiotropic molecule that exerts its biological activities via binding to the GH receptor (GHR), a type I cytokine receptor expressed by a wide variety of cell types. It is coupled to signaling cascades that include JAK/STAT, PI3-Kinase, and MAP kinase (24). Proteolytic cleavage near the transmembrane domain releases a circulating high-affinity binding form of the receptor termed GH-binding protein (GHBP) (25, 26). It is estimated that half of the circulating hGH may be associated with GHBP (27, 28). The growth-promoting effects of hGH may occur directly, or indirectly through the stimulated production of IGF-I (24). GH may also have an immunomodulatory role through its effects on the proliferation and activities of B cells, T cells, NK cells, macrophages, and neutrophils (29). In addition, GH can act directly on various cell types to induce lipolysis, lactation, amino acid uptake, and protein synthesis (24, 29, 30).

Overproduction of hGH can lead to acromegaly, a disorder characterized by enlargement of the hands, feet, and facial features, as well as enhanced risk of cardiovascular disease, respiratory disease, and malignancy (31). Overproduction prior to puberty can result in gigantism (31). hGH deficiency in adults may lead to increases in cardiovascular risk, body fat, and insulin sensitivity, as well as decreased muscle mass and bone density (32, 33). In children, hGH-deficiency may also contribute to slow growth rates and short stature (33).

The Quantikine® Human Growth Hormone Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Growth Hormone in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Growth Hormone and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Growth Hormone 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 naturally occurring human Growth Hormone.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Growth Hormone has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Growth Hormone present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Growth Hormone 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 Growth Hormone 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 #	CATALOG # DGH00	CATALOG # SGH00	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Growth Hormone Microplate	893020	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Growth Hormone.	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 Growth Hormone Conjugate	893021	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human Growth Hormone conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Growth Hormone Standard	893022	1 vial	6 vials	Recombinant human Growth Hormone in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-57	895207	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6-15	895244	1 vial	6 vials	21 mL/vial of a buffered protein base 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.

DGH00 contains sufficient materials to run an ELISA on one 96 well plate.
SGH00 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.
This kit is also available in a PharmPak (R&D Systems®, Catalog # PDGH00). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. 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 Growth Hormone Controls (optional; R&D Systems®, Catalog # QC99).

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 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 heparin or EDTA 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.*
Grossly hemolyzed samples are not suitable for use in this assay.

REAGENT PREPARATION

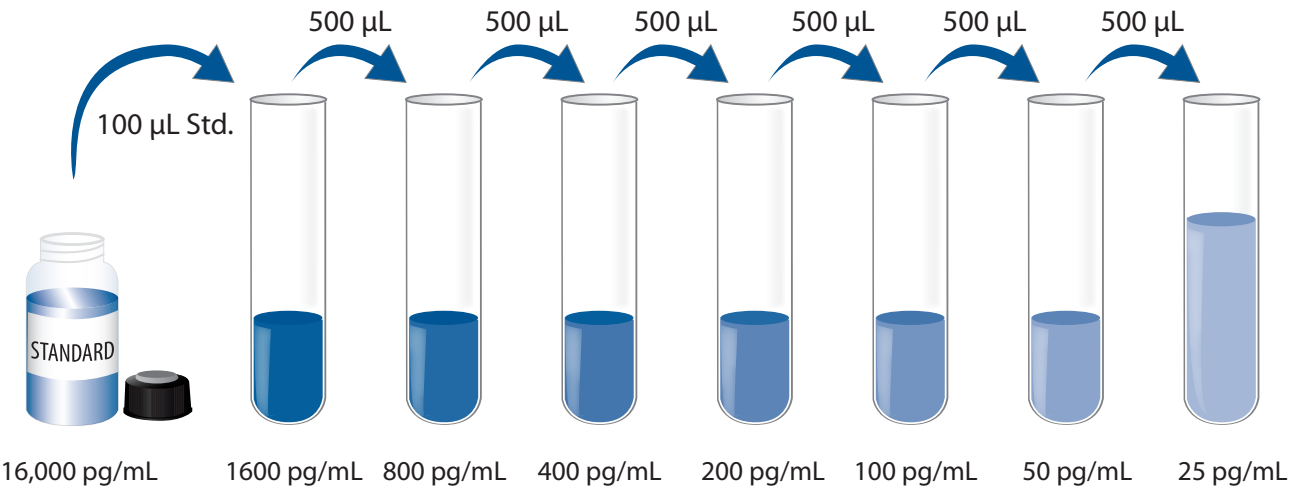
Bring all reagents to room temperature before use.

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.

Human Growth Hormone Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Growth Hormone Standard with deionized or distilled water. This reconstitution produces a stock solution of 16,000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6-15 (*for serum/plasma samples*) into the 1600 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 1600 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 RD1-57 to each well.
4. Add 50 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 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 Growth Hormone Conjugate to each well. Cover with a new adhesive strip. 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 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.).

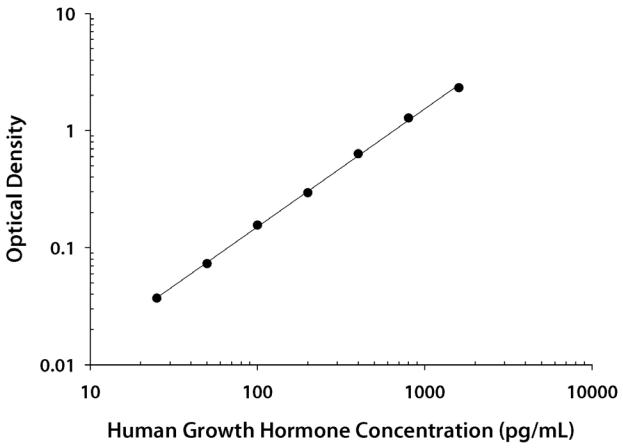
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points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

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

TYPICAL DATA

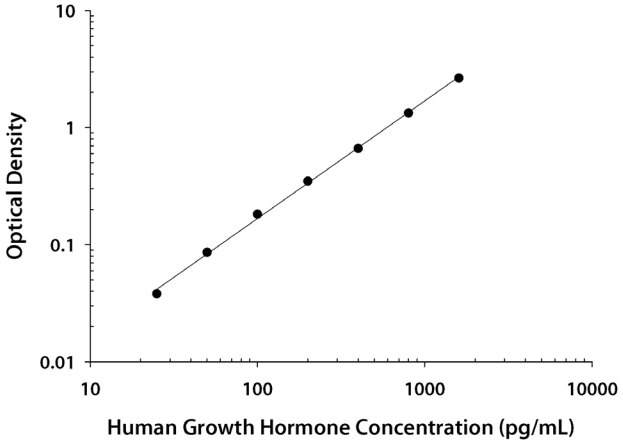
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.028 0.028	0.028	—
25	0.064 0.065	0.065	0.037
50	0.100 0.101	0.101	0.073
100	0.184 0.184	0.184	0.156
200	0.304 0.342	0.323	0.295
400	0.655 0.668	0.662	0.634
800	1.303 1.310	1.307	1.279
1600	2.241 2.453	2.347	2.319

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.027 0.028	0.028	—
25	0.065 0.067	0.066	0.038
50	0.112 0.115	0.114	0.086
100	0.208 0.212	0.210	0.182
200	0.369 0.384	0.377	0.349
400	0.678 0.701	0.690	0.662
800	1.291 1.427	1.359	1.331
1600	2.618 2.716	2.667	2.639

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)	201	595	1091	209	623	1152
Standard deviation	9.4	18.8	26.0	18.2	45.6	63.6
CV (%)	4.7	3.2	2.4	8.7	7.3	5.5

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)	197	586	1092	215	639	1189
Standard deviation	8.0	13.8	29.8	20.2	49.5	82.2
CV (%)	4.1	2.4	2.7	9.4	7.7	6.9

RECOVERY

The recovery of human Growth Hormone spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	100	97-105%
Serum (n=4)	105	98-111%
EDTA plasma (n=4)	108	101-117%
Heparin plasma (n=4)	107	99-117%

SENSITIVITY

Eighty assays were evaluated and the minimum detectable dose (MDD) of human Growth Hormone ranged from 0.64-7.18 pg/mL. The mean MDD was 2.10 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.

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human Growth Hormone were serially 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	102	106	100	106
	Range (%)	102-103	104-108	88-107	105-110
1:4	Average % of Expected	103	111	104	113
	Range (%)	101-108	106-115	94-113	107-119
1:8	Average % of Expected	102	114	106	111
	Range (%)	100-104	111-116	94-116	108-115
1:16	Average % of Expected	102	109	98	103
	Range (%)	92-111	90-117	85-114	81-118

CALIBRATION

This immunoassay utilizes a highly purified *E. coli*-expressed recombinant human Growth Hormone produced at R&D Systems® that is directly calibrated to the NIBSC/WHO 2nd International Standard 98/574, a recombinant DNA-derived human Growth Hormone.

The NIBSC/WHO International Standard (80/505), derived from human pituitary, was also evaluated in this immunoassay.

To convert sample values obtained with the Quantikine® kit to approximate NIBSC (80/505) units, use the equation below.

NIBSC/WHO (80/505) approximate value (U/mL) = 1.39 x Quantikine® Human Growth Hormone value (pg/mL)

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Growth Hormone in this assay. No medical histories were available for the donors used in this study.

Females	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=18)	550	100	29.3-4360
EDTA plasma (n=18)	648	100	30.2-5936
Heparin plasma (n=18)	641	100	38.1-5392

Males	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=17)	62.0	83	ND-120.5
EDTA plasma (n=17)	56.4	86	ND-99.2
Heparin plasma (n=17)	58.1	86	ND-109.6

ND=Non-detectable

Cell Culture Supernates - Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal bovine 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 supernates were removed and assayed for levels of human Growth Hormone. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human Growth Hormone.

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 recombinant human Growth Hormone control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

β-ECGF
EGF
FGF acidic
FGF basic$_{146}$
FGF-4
FGF-5
FGF-6
FGF-9
FGF-10
FGF-18
Flt-3 Ligand
G-CSF
Growth Hormone (20 kDa)
GM-CSF
HB-EGF
HGF
HRG-α
IGF-I
IGF-II
KGF (FGF-7)
M-CSF
MSP
MSPβ
β-NGF
PDGF-AA
PDGF-AB
PDGF-BB
PD-ECGF
PlGF
Prolactin
VEGF$_{121}$
VEGF$_{165}$
VEGF/PlGF
VEGF-D
VEGF R1

Recombinant mouse:

FGF-8b
FGF-8c
Flt-3 Ligand
G-CSF
GM-CSF
M-CSF
PlGF-2
VEGF$_{120}$
VEGF$_{164}$

Recombinant rat:

GM-CSF
β-NGF
PDGF-BB

Recombinant porcine:

GM-CSF
PDGF

Natural proteins:

human CG
bovine FGF acidic
bovine FGF basic
human FSH
bovine GH
human GH (5 kDa)
human LH
human PDGF
human TSH

Recombinant human and recombinant rat Growth Hormone R interfere at concentrations > 1 ng/mL.

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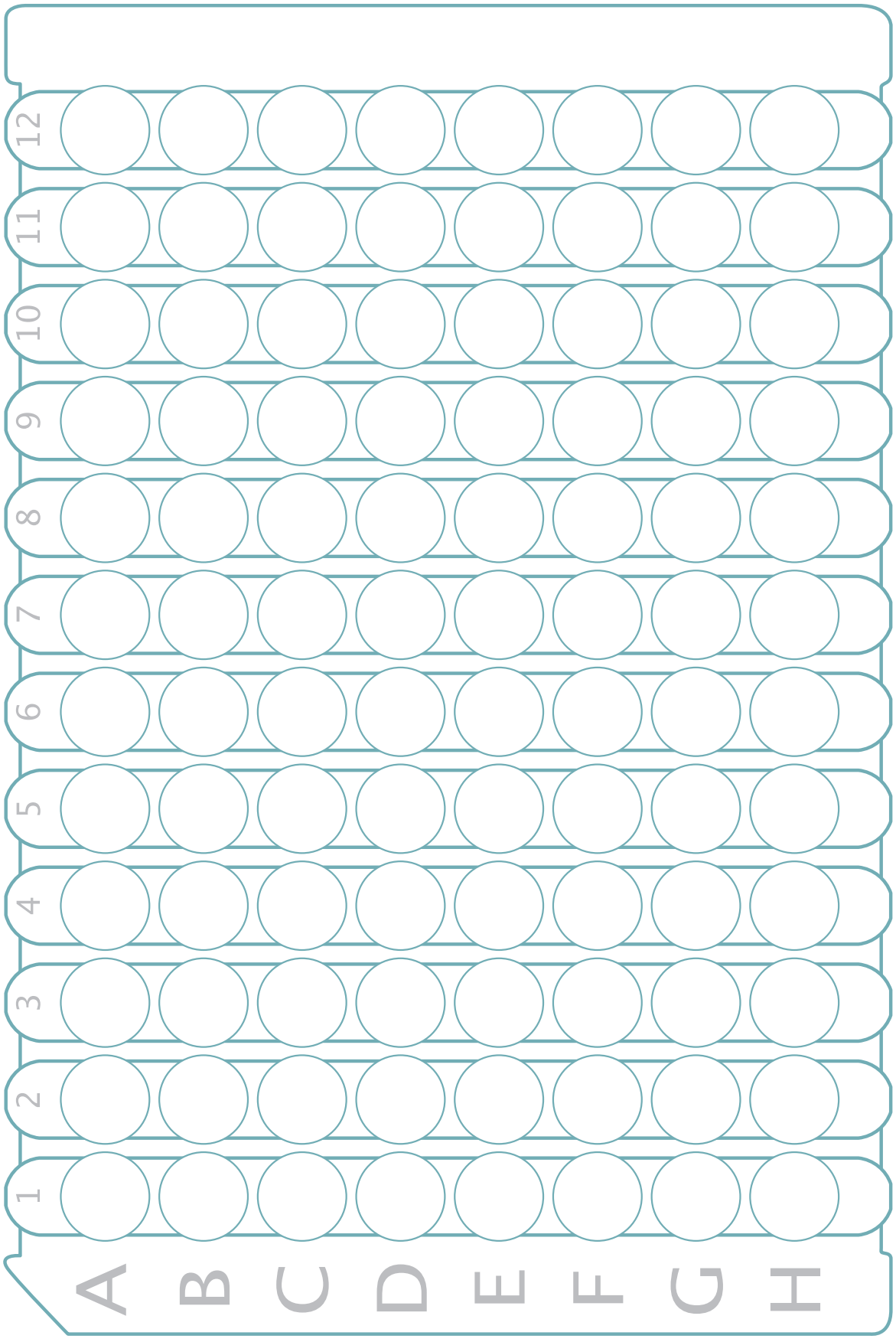
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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