Quantikine® ELISA

Human Flt-3 Ligand/FLT3L Immunoassay

Catalog Number DFK00

For the quantitative determination of human Flt-3 Ligand 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 Flt-3 Ligand is a 30 to 35 kDa, type I (external N-terminus) transmembrane glycoprotein. Flt-3 Ligand is not closely related to other cytokines, but shows some homology to SCF and M-CSF (1-3). Although amino acid (aa) sequence identity between Flt-3 Ligand, SCF, and M-CSF is less than 15% (2), the positions of four cysteine residues and of four α-helical regions are conserved in these three proteins (1, 2, 4). Flt-3 Ligand is synthesized as a 235 aa residue precursor, with a 156 aa residue extracellular domain, a 23 aa residue transmembrane segment, and a 30 aa residue cytoplasmic tail (1). Within the molecule, there are three intrachain disulfide bonds arranged around four α-helices (1, 4). When human Flt-3 Ligand is compared to mouse Flt-3 Ligand, the aa sequence identity is about 72% and each ligand binds the corresponding receptor of the other species (1, 2). Alternative splice events occur within the Flt-3 Ligand gene, resulting in the expression of both soluble and membrane-bound forms (1, 5, 6). The membrane-bound forms appear to be of two types, one which engages in cell-cell interaction and a second whose function is to be specifically cleaved to create a soluble form of Flt-3 Ligand (5, 6). Although alternative splicing can also give rise directly to soluble Flt-3 Ligand, it is reported that the proteolytically processed molecule is more naturally abundant and is 10 times more active (5). As a soluble form, Flt-3 Ligand exists as a noncovalently-linked homodimer (4) and has been detected in normal blood at pg/mL concentrations (7). The membrane-bound form of Flt-3 Ligand is far more prevalent than any soluble form and thus seems to be the most biologically important form (5, 6). Cells known to express Flt-3 Ligand include keratinocytes (8), fibroblasts (8, 9), and T-cells (5).

The receptor for human Flt-3 Ligand was cloned from a pre-B cell line and found to be a member of the Class III tyrosine kinase receptor (or TKR) family (3, 10, 11). The human receptor has been variously termed STK-1 (stem cell tyrosine kinase-1) (11) or human Flt-3/Flk-2 (*fms*-like tyrosine kinase-3/fetal liver kinase-2) (10) after the designations previously given to the mouse homolog that was independently isolated and named by two laboratories (12, 13). Human Flt-3/Flk-2 is a type I transmembrane glycoprotein approximately 160 kDa in size (10, 11). The mature molecule is 970 aa residues in length, with a 518 aa residue extracellular region, a 21 aa residue transmembrane segment, and a 431 aa residue cytoplasmic domain. As with all Class III TKRs, the extracellular region contains five Ig-like domains which, in their entirety, are approximately 20% identical to the extracellular regions of the Class III receptors for SCF and M-CSF (3, 10, 11). In the mouse, Flt-3/Flk-2 is about the same size as human Flt-3/Flk-2 and, mouse to human, there is approximately 86% identity in overall aa sequence (10, 12). Following ligand binding, monomeric Flt-3/Flk-2 forms a non-covalently linked homodimer that binds Flt-3 Ligand with a Kd of 100 to 500 pM (14). Cells reported to express Flt-3/Flk-2 include immature thymocytes (13), monocytes (10), primitive B-cell progenitors (15), bile duct epithelial cells (16), and $CD34^+$ stem cells that are destined to become more committed progenitor cells (17, 18).

Functionally, Flt-3 Ligand has been implicated in many early events surrounding lymphopoiesis and myelopoiesis. During lymphopoiesis, Flt-3 Ligand has been suggested to promote the self-renewal of thymic progenitors (19), prolong the survival of primitive B cell progenitors (20), promote the proliferation of early B cell progenitors (21), and with IL-7, induce the differentiation of B cell lineage cell types (22). During myelopoiesis, Flt-3 Ligand apparently has positive effects on CFU-GM development and subsequent monocyte differentiation (23, 24). In contrast, Flt-3 Ligand has limited or no effect on either mast cell development or megakaryopoiesis (25, 26).

The Quantikine® Human Flt-3 Ligand/FLT3L Immunoassay is a 4-4.5 hour solid phase ELISA designed to measure soluble human Flt-3 Ligand in cell culture supernates, serum, and plasma. It contains NS0-expressed recombinant human Flt-3 Ligand and antibodies raised against the recombinant factor. This immunoassay has been shown to quantitate recombinant Flt-3 Ligand accurately. Results obtained measuring natural human Flt-3 Ligand showed dose-response curves that were parallel to the standard curves obtained using the recombinant Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human Flt-3 Ligand.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Flt-3 Ligand has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Flt-3 Ligand present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Flt-3 Ligand 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 Flt-3 Ligand 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 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 Flt-3 Ligand Microplate	890556	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Flt-3 Ligand.	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 Flt-3 Ligand Conjugate	890557	21 mL of a polyclonal antibody specific for human Flt-3 Ligand conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Flt-3 Ligand Standard	890558	Recombinant human Flt-3 Ligand in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	11 mL/vial 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 RD6-11	895489	21 mL of a 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 Flt-3 Ligand Controls (optional; R&D Systems®, Catalog # QC21)

PRECAUTIONS

Calibrator Diluent RD6-11 contains sodium azide which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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 SDS 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

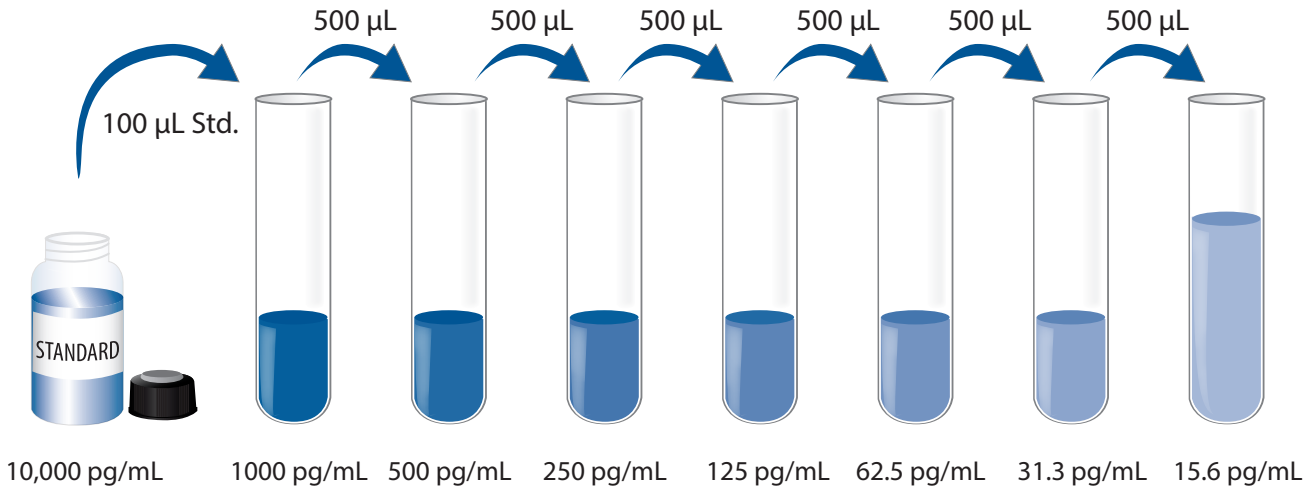
Bring all reagents to room temperature before use.

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 480 mL of 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 Flt-3 Ligand Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Flt-3 Ligand Standard with deionized or distilled water. This reconstitution produces a stock solution of 10,000 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 Calibrator Diluent RD5R (*for cell culture supernate samples*) or Calibrator Diluent RD6-11 (*for serum/plasma samples*) into the 1000 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 1000 pg/mL standard serves as the high standard. The appropriate calibrator diluent serves as the zero standard (0 pg/mL).



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ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, controls, and samples 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 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 samples and standards 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 Flt-3 Ligand Conjugate to each well. Cover with a new adhesive strip.
   For Cell Culture Supernate Samples: Incubate for 1.5 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 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.).

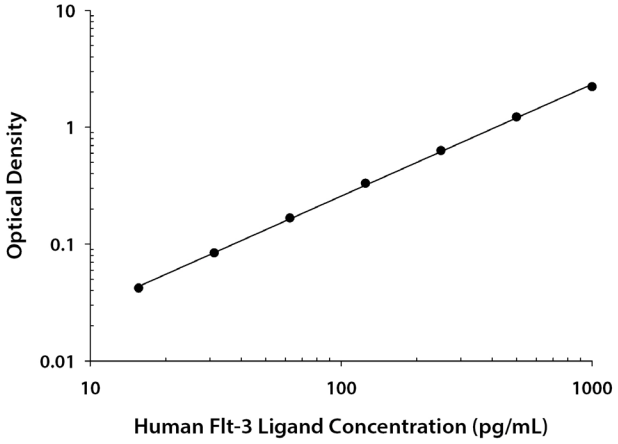
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human Flt-3 Ligand 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

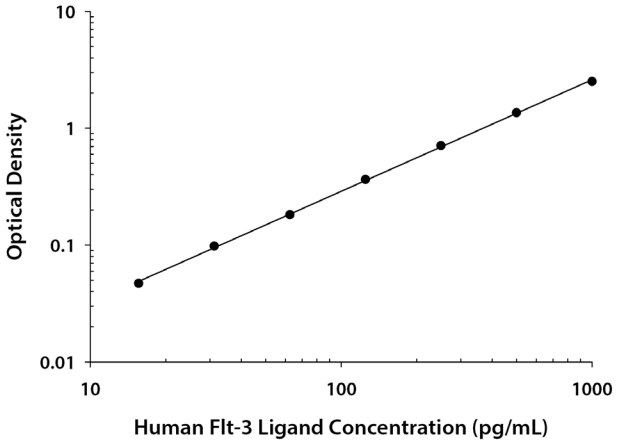
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATES ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.036 0.034	0.035	—
15.6	0.078 0.076	0.077	0.042
31.3	0.118 0.121	0.120	0.085
62.5	0.208 0.201	0.204	0.169
125	0.361 0.382	0.372	0.337
250	0.675 0.660	0.668	0.633
500	1.259 1.255	1.257	1.222
1000	2.255 2.246	2.250	2.215

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.045 0.045	0.045	—
15.6	0.092 0.091	0.092	0.047
31.3	0.144 0.142	0.143	0.098
62.5	0.229 0.225	0.227	0.182
125	0.421 0.396	0.408	0.363
250	0.738 0.772	0.755	0.710
500	1.436 1.363	1.400	1.355
1000	2.579 2.531	2.555	2.510

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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)	65.7	214	421	72.8	224	429
Standard deviation	1.2	4.1	9.3	6.7	13.8	30.5
CV (%)	1.8	1.9	2.2	9.2	6.2	7.1

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)	69.5	230	453	78.2	241	462
Standard deviation	1.9	5.2	6.2	8.7	14.9	33.1
CV (%)	2.7	2.3	1.4	11.1	6.2	7.2

RECOVERY

The recovery of human Flt-3 Ligand 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	92-118%
Serum (n=5)	100	91-117%
EDTA plasma (n=5)	101	90-119%
Heparin plasma (n=5)	100	88-112%
Citrate plasma (n=5)	101	94-110%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human Flt-3 Ligand 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	101	105	100	105	103
	Range (%)	91-106	100-110	90-104	103-108	96-105
1:4	Average % of Expected	101	105	102	108	102
	Range (%)	93-108	101-107	95-106	103-111	90-108
1:8	Average % of Expected	100	105	103	106	100
	Range (%)	89-108	99-110	96-108	99-112	94-105
1:16	Average % of Expected	99	101	103	104	105
	Range (%)	87-107	93-109	92-109	93-113	99-110

SENSITIVITY

The minimum detectable dose (MDD) of human Flt-3 Ligand is typically less than 7 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.

CALIBRATION

This immunoassay is calibrated against a highly purified NS0-expressed recombinant human Flt-3 Ligand produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Flt-3 Ligand 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=101)	95	48-174	26
EDTA plasma (n=37)	97	62-145	19
Heparin plasma (n=37)	106	69-154	21
Citrate plasma (n=37)	87	59-131	19

Cell Culture Supernates - Human peripheral blood mononuclear cells (5 x 10^6 cells/mL) were cultured in RPMI supplemented with 5% fetal bovine serum, 50 µM β-mercaptoethanol, 2 mM L-glutamine, 100 U/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 Flt-3 Ligand.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated cells	ND	ND
Stimulated cells	58.4	50.4

ND-Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human Flt-3 Ligand.

The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors prepared at 50 ng/mL in a mid-range recombinant human Flt-3 Ligand control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

EGF
Epo
FGF acidic
FGF basic
Flt-1
G-CSF
GM-CSF
HB-EGF
HGF
KGF/FGF-7
M-CSF
SCF
TGF-β1
TGF-β3
TGF-β RII
VEGF

Recombinant mouse:

Flt-3 Ligand

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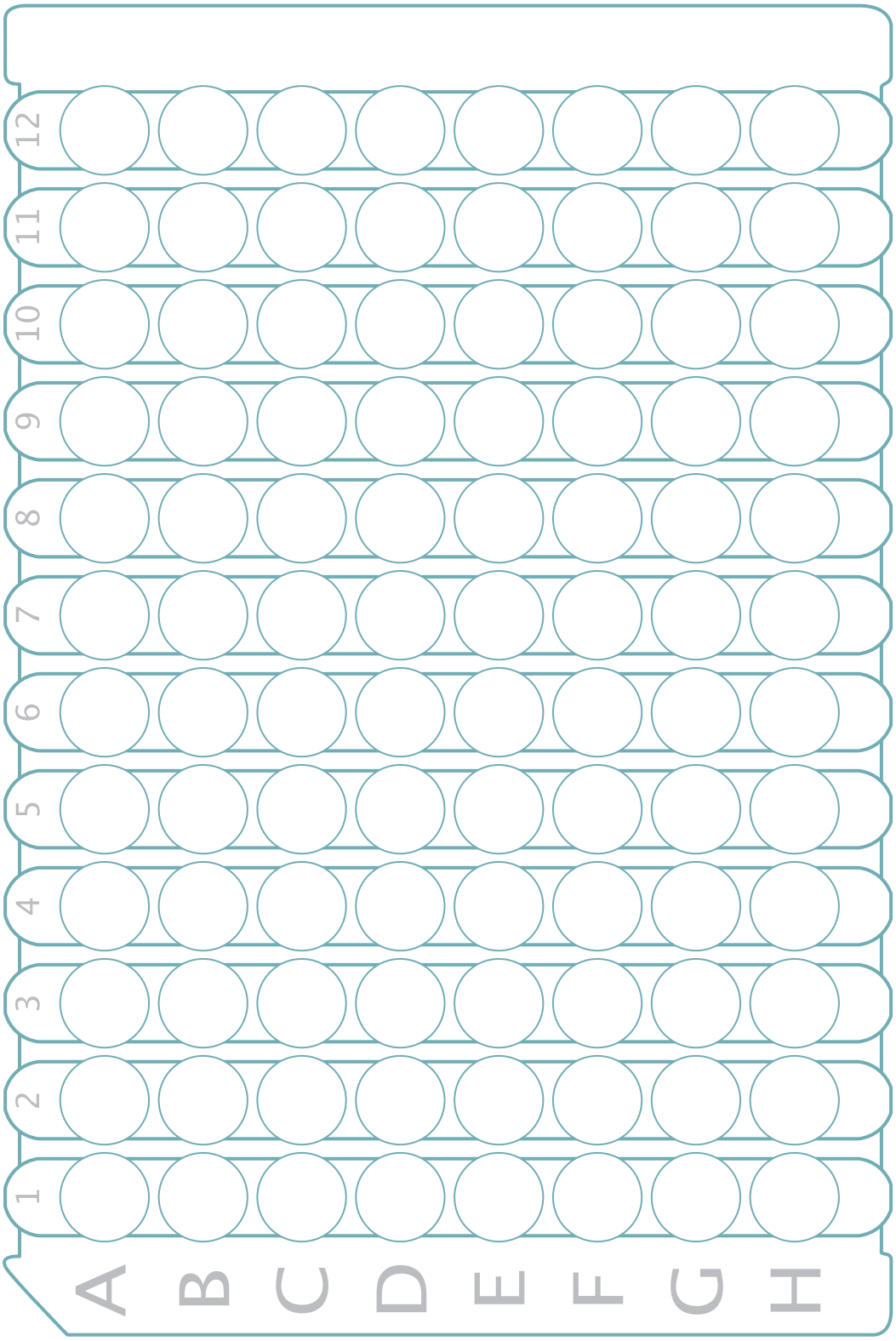
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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