Quantikine® ELISA

Human SCF Immunoassay

Catalog Number DCK00

For the quantitative determination of human Stem Cell Factor (SCF) 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

Mice carrying gene mutations in either the dominant white spotting locus (W) or the steel locus (Sl) develop similar phenotypic abnormalities, including deficiencies in hematopoiesis, melanogenesis, and gametogenesis (1). A transmembrane tyrosine kinase proto-oncogene, designated *c-kit* was mapped to the W locus (2-4). The gene encoding a novel cytokine, the ligand for the *c-kit* tyrosine kinase receptor, was independently mapped by several groups of investigators to the Sl locus (5-10). Several names for this pleiotropic cytokine have been suggested, including stem cell factor (SCF), c-kit ligand (KL), mast cell growth factor (MGF), or steel-factor (SLF). For reviews on SCF, see references 11-15.

Initially, cDNA sequences for human and mouse SCF were discovered that encoded a transmembrane protein composed of a 25 amino acid (aa) residue signal peptide, a 189 aa extracellular domain, a 23 aa hydrophobic transmembrane span, and a 36 aa cytoplasmic segment (5, 8, 16). Subsequent analysis in the mouse revealed an alternative splice variant that deletes an entire exon, resulting in a transmembrane molecule 28 aa shorter in the extracellular domain (17, 18). Human and mouse systems are now both known to contain the 248 aa and 220 aa alternatively spliced forms of SCF (19). Both the larger 45 kDa form (also called KL-1) and the smaller 32 kDa form (also called KL-2) are cleaved to produce soluble factors. Cleavage of KL-1 gives rise to a 31 kDa soluble form. The splice variant KL-2 lacks the proteolytic cleavage site used to generate soluble KL-1, but uses a site that is cleaved with lower efficiency to generate a 23 kDa soluble molecule (17, 18). Cells identified as possible sources for SCF include fibroblasts, liver cells, Sertoli cells, endothelial cells, neurons, macrophages, oocytes, Schwann cells, cytotrophoblast cells, stratified squamous epithelium and numerous carcinoma cell lines (20-29). Expression of KL-1 and KL-2 seems to be tissue-specific. KL-1 is associated with fibroblasts, brain and thymus while KL-2 is found in the spleen, testis, placenta and cerebellum (17). Both the soluble and the transmembrane forms of SCF have growth factor activities. Since mouse mutations (Sld) capable of encoding only the soluble truncated form of SCF, lacking both the transmembrane and cytoplasmic domains, exhibit phenotypic defects almost identical to those mutations in which no SCF protein is produced, the membrane-bound form must be important in mediating cell-cell adhesion and interaction and must have a critical biological role in the intact organism (24, 30-33).

Native soluble SCF is a heavily N- and O-glycosylated protein that exists as a non-covalently associated dimer in solution. All four cysteine residues of SCF monomers are involved in intramolecular disulfide bonds (34). Recombinant soluble SCF produced in *E. coli* is biologically active in *in vitro* bioassays, suggesting that glycosylation of the soluble form is not required for bioactivity *in vitro*. Mouse or rat soluble SCF is highly homologous to human soluble SCF (approximately 80% sequence identity). Whereas both rat and mouse SCF are active on human cells, the human protein is 800 fold less active on mouse or rat cells (16). As predicted by the phenotypes of various Sl and W mutations, SCF has biological activities in three migratory cell lines including melanocytes (35), primordial germ cells (31), and hematopoietic progenitor cells during embryonal development and postnatal life. Although phenotypic defects in the nervous system have not been described for the Sl or W mutations to date, a complex pattern of SCF expression in the nervous system has been observed (36, 37).

The multiple hematologic defects (macrocytic anemia, mast cell and bone marrow CFU-S deficiencies) observed for mice carrying Sl mutations and the results obtained from *in vivo* and *in vitro* experiments using purified SCF are consistent with the suggestion that SCF is a pleiotropic growth factor with diverse hematopoietic target cells, including early progenitor cells (6, 11-13, 33, 38-40). SCF can stimulate the proliferation of mature and immature mast cells *in vitro* and *in vivo* (41, 42). In cultures of cord blood-derived immature mast cells, SCF stimulates the proliferation and differentiation of immature mast cells and their progenitors during the early stages of culture. At later stages the effect of SCF is merely to maintain the survival of immature mast cells (43). SCF has also been demonstrated to facilitate the release of mast cell inflammatory mediators, either in the presence or absence of IgE-associated stimuli (44, 45). On purified primitive hematopoietic precursors purified from mice (46) or from humans (47, 48), SCF acts in a synergistic manner with various growth factors, such as IL-3, IL-6, IL-11, GM-CSF, G-CSF, and Epo to induce myeloid and erythroid lineage colony formation (49-52). In concert with IL-3, SCF has now been shown to serve as a growth and differentiation factor for the pluripotent $CD34^+/4\text{-}HC^{res}$ (pre-CFU) stem cell (53-55). SCF has been reported to protect human marrow progenitors from high radiation doses (56) and to expand the primitive cellular components of bone marrow (pre-CFU-S, BFU-E and MK and CFU-MK, GEMM, and GM) following transplantation and thus accelerating recovery of bone marrow function (57, 58). Other SCF activities not involving hematopoiesis or mast cell physiology include proliferative activity for type A (or primitive) spermatogonia (59), trophic and neurite-inductive activity in a subpopulation of dorsal root ganglia neurons (60) and, in conjunction with IL-7, a clonal expansive activity for $CD45^+$ B cells (61).

The Quantikine® SCF Immunoassay is a 4.5 hour solid phase ELISA designed to measure human SCF levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human soluble SCF (KL-1 variant) and antibodies raised against this recombinant factor. Results obtained for natural human SCF samples 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 SCF.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human SCF has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any SCF present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human SCF 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 SCF 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 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 SCF Microplate	890105	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human SCF.	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 SCF Conjugate	890106	21 mL of a polyclonal antibody specific for human SCF conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human SCF Standard	890107	Recombinant human SCF in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1-1	895143	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-5	895485	21 mL of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Calibrator Diluent RD6E	895017	21 mL of animal serum 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.
- 500 mL graduated cylinder.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Test tubes for dilution of standards.
- Human SCF controls (optional; R&D Systems®, Catalog # QC20).

PRECAUTIONS

Some components of this kit contain 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 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.

Note: *Culture media supplemented with human serum or plasma may contain high levels of stem cell factor.*

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

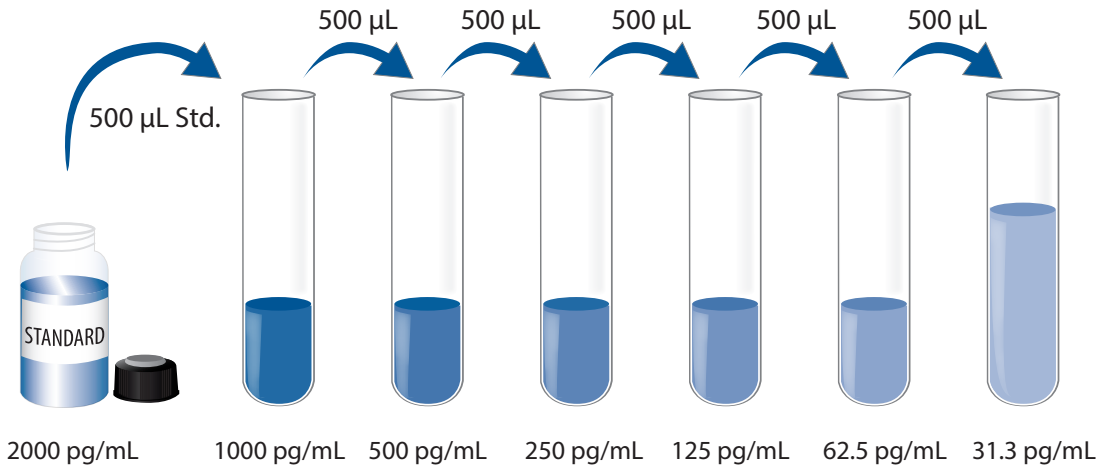
Bring all reagents to room temperature before use.

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 SCF Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human SCF Standard with Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6E (*for serum/plasma samples*). This reconstitution produces a stock solution of 2000 pg/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 500 µL of the Calibrator Diluent RD5-5 (*for cell culture supernate samples*) or Calibrator Diluent RD6E (*for serum/plasma samples*) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Human SCF Standard (2000 pg/mL) 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 RD1-1 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 SCF 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 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.).

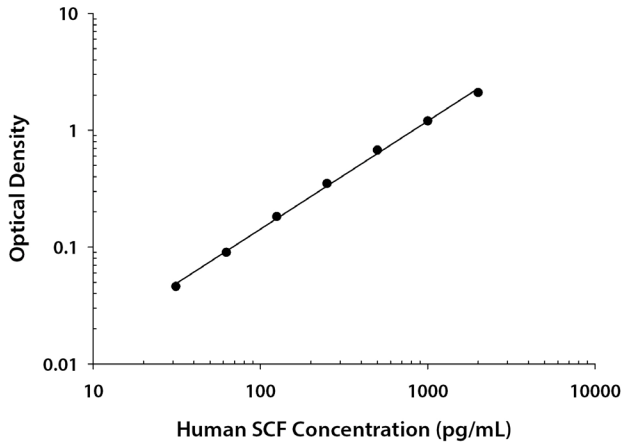
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points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

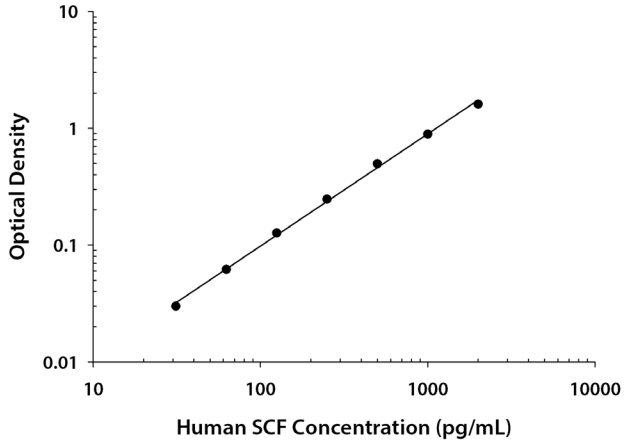
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.020 0.020	0.020	—
31.3	0.065 0.066	0.066	0.046
62.5	0.107 0.112	0.110	0.090
125	0.201 0.204	0.202	0.182
250	0.368 0.371	0.370	0.350
500	0.696 0.696	0.696	0.676
1000	1.222 1.223	1.222	1.202
2000	2.117 2.128	2.122	2.102

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.017 0.018	0.018	—
31.3	0.047 0.049	0.048	0.030
62.5	0.080 0.081	0.080	0.062
125	0.145 0.145	0.145	0.127
250	0.264 0.266	0.265	0.247
500	0.492 0.536	0.514	0.496
1000	0.906 0.909	0.908	0.890
2000	1.621 1.629	1.625	1.607

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)	153	709	1427	151	729	1552
Standard deviation	3.6	11.6	61.0	12.1	45.8	125
CV (%)	2.4	1.6	4.3	8.0	6.3	8.1

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)	139	655	1419	142	703	1574
Standard deviation	5.5	12.9	54.0	14.6	36.0	94.0
CV (%)	4.0	2.0	3.8	10.3	5.1	6.0

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media	85	75-93%
Serum	99	87-111%
EDTA plasma	98	82-110%
Heparin plasma	99	88-116%
Citrate plasma	94	84-112%

SENSITIVITY

The minimum detectable dose of human SCF is typically less than 9.0 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 linearity of the assay, the following biological samples containing and/or spiked with human SCF were diluted with the appropriate calibrator diluent and then assayed.

Sample	Dilution	Observed (pg/mL)	Expected (pg/mL)	$\frac{\text{Observed}}{\text{Expected}} \times 100$
Cell culture media	Neat	1031	———	———
	1:2	510	516	99%
	1:4	288	258	112%
	1:8	149	129	116%
Serum	Neat	1421	———	———
	1:2	714	710	101%
	1:4	362	355	102%
	1:8	183	178	103%
	1:16	79	89	89%
EDTA plasma	Neat	1391	———	———
	1:2	682	696	98%
	1:4	329	348	95%
	1:8	177	174	102%
	1:16	77	87	89%
Heparin plasma	Neat	1439	———	———
	1:2	746	720	104%
	1:4	372	360	103%
	1:8	177	180	98%
	1:16	100	90	111%
Citrate plasma	Neat	1371	———	———
	1:2	708	686	103%
	1:4	350	343	102%
	1:8	180	172	105%
	1:16	74	86	86%

CALIBRATION

This immunoassay is calibrated against highly purified *E. coli*-expressed recombinant human soluble SCF produced at R&D Systems®.

The NIBSC/WHO Reference Reagent of SCF 91/682, which is intended as a potency standard, was evaluated in this kit. The dose-response curve of the reference material parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human SCF kit to approximate NIBSC 91/682 arbitrary units, use the equation below.

NIBSC (91/682) approximate value (U/mL) = 0.0014 x Quantikine® Human SCF value (pg/mL)

SAMPLE VALUES

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

Sample Type	Mean (pg/mL)	Range (pg/mL)
Serum (n=40)	984	558-1441
EDTA plasma (n=30)	1029	752-1500
Heparin plasma (n=30)	887	622-1183
Citrate plasma (n=30)	865	629-1187

SPECIFICITY

This assay recognizes natural and recombinant human SCF.

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

Recombinant human:

CNTF
EGF
Epo
G-CSF
GM-CSF
IL-1α
IL-1β
IL-2
IL-3
IL-4
IL-5
IL-6
IL-7
IL-8
LIF
SCF R
TGF-β1
TNF-α

The following factors cross-react in this assay:

Recombinant Factor	% Cross-reactivity
Canine SCF	15.6
Feline SCF	16.1
Mouse SCF	3.5
Rat SCF	1.0

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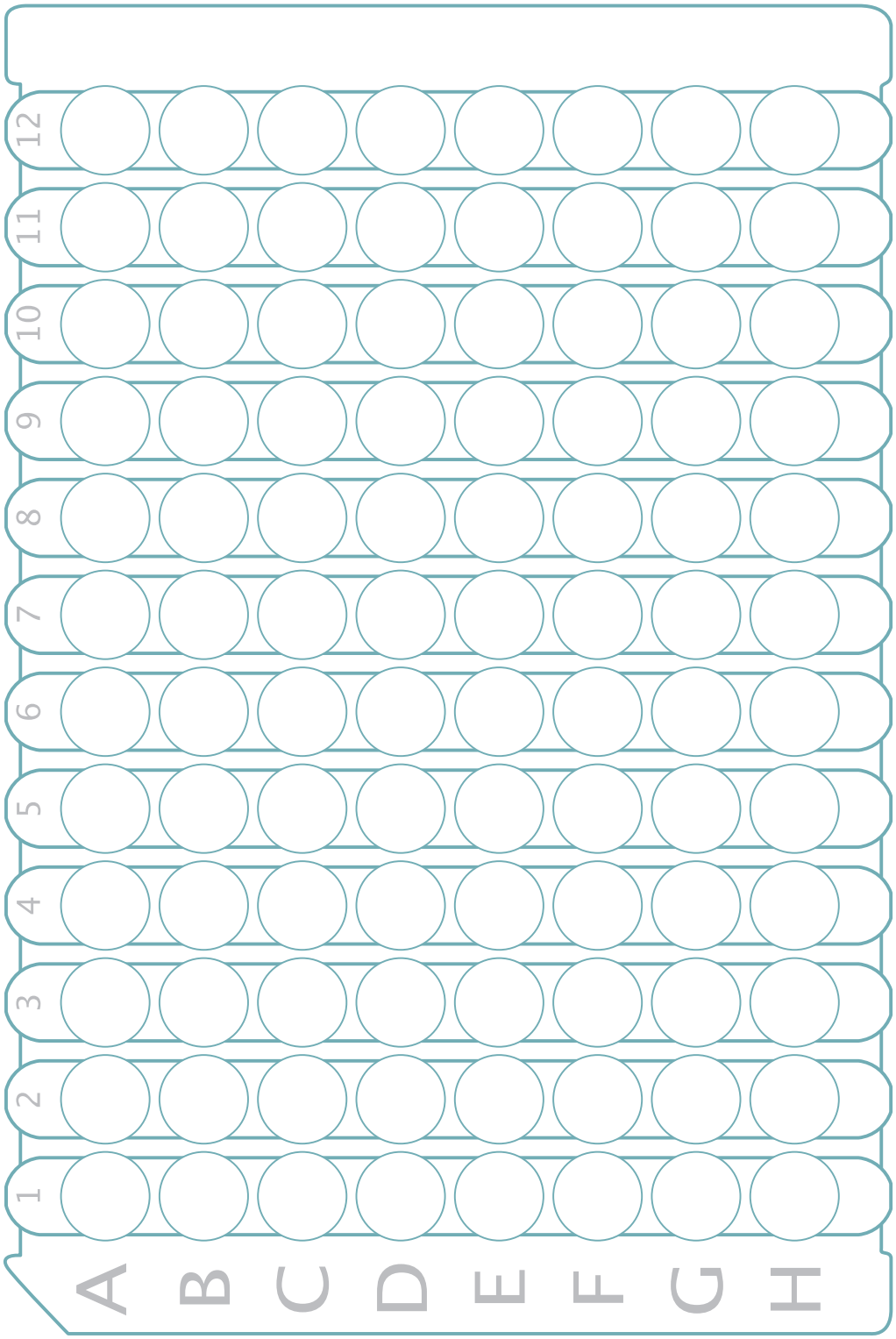
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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