Quantikine® ELISA

Human CD117/c-kit Immunoassay

Catalog Number DSCR00

For the quantitative determination of human CD117/c-kit/SCF R 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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MANUFACTURED AND DISTRIBUTED BY:

USA & Canada | R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413, USA
TEL: (800) 343-7475 (612) 379-2956 FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

UK & Europe | R&D Systems Europe, Ltd.
19 Barton Lane, Abingdon Science Park, Abingdon OX14 3NB, UK
TEL: +44 (0)1235 529449 FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

China | R&D Systems China Co., Ltd.
24A1 Hua Min Empire Plaza, 726 West Yan An Road, Shanghai PRC 200050
TEL: +86 (21) 52380373 FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

INTRODUCTION

C-kit (the gene product of the c-kit proto-oncogene), also known as CD117 and stem cell factor receptor (SCF R), is the cellular receptor for stem cell factor (SCF), also named c-kit ligand, mast cell growth factor, and steel factor. This receptor-ligand system plays essential roles in germ cell development, melanogenesis, hematopoiesis, and oncogenesis (1-6). The human c-kit cDNA encodes a 972 amino acid (aa) precursor transmembrane protein with a 25 aa signal peptide, a 495 aa extracellular domain, a 23 aa residue transmembrane segment, and a 429 aa residue cytoplasmic domain (6, 7). c-kit is a member of the type III subfamily of receptor tyrosine kinases (RTK) that also includes M-CSF, Flt-3, PDGF, and VEGF. All class III RTKs are characterized by the presence of five immunoglobulin-like (Ig like) domains in their extracellular region and a split kinase domain in their intracellular region. SCF homodimer-binding induces receptor homodimerization and signal transduction cascades that include the activation of PLC-γ, PI 3-Kinase, Ras/MAP Kinase, and Src (8-12). The first three Ig-like motifs appear to mediate binding with SCF (13, 14).

SCF/c-kit plays critical roles in hematopoietic development. As such, c-kit is expressed in many cell types of the hematopoietic hierarchy including progenitors from lymphoid, myeloid, erythroid, and megakaryocyte lineages, as well as some mature NK cells (4, 15). It is also expressed in an array of both murine and human hematopoietic cell lines (4, 15). Other cell and tissue types expressing c-kit include germ cells, melanocytes, glial cells, vascular smooth muscle cells, placenta, and epithelial cells of the breast, kidney, and parotid gland (16-18). c-kit can be proteolytically shed from the cell surface, potentially by tumor necrosis factor α-converting enzyme (TACE; ADAM17) (19). Soluble forms of c-kit may act as natural, competitive antagonists for the transmembrane receptor, and have also been shown to mobilize hematopoietic stem cells from bone marrow (20-22). *In vitro*, treatment with phorbol ester or elevations of calcium can lead to the production of soluble c-kit in cell culture supernates, and it has been detected in human plasma, follicular fluid, and cerebrospinal fluid (20, 23-28).

Altered levels and mutations in c-kit have been described in several types of cancer including lung, breast, gastrointestinal stromal, and germ cell tumors, and the receptor has been implicated as a target for cancer therapies (3, 29-34). Levels of the soluble form of the receptor, specifically, may be altered in patients with hematologic malignancies, central nervous system germinoma, gastrointestinal tumors, atopic dermatitis, systemic lupus erythematosus, and graft-versus-host disease (28, 35-41).

The Quantikine Human CD117/c-kit Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human CD117/c-kit/SCF R in cell culture supernates, serum, and plasma. It contains *Sf* 21-expressed recombinant human CD117/c-kit/SCF R and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human CD117/c-kit/SCF R 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 CD117/c-kit/SCF R.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human CD117/c-kit/SCF R has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any CD117/c-kit/SCF R present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human c-kit 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 CD117/c-kit/SCF R 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 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 SCF R Microplate	892864	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody against CD117/c-kit/SCF R.	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 R Conjugate	892865	21 mL of monoclonal antibody against CD117/c-kit/SCF R conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human SCF R Standard	892866	500 ng of recombinant human CD117/ c-kit/SCF R in a buffered protein base with preservatives; lyophilized.	
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5P Concentrate	895151	21 mL of a concentrated buffered protein base with preservatives. *Use diluted 1:3 for cell culture supernate samples. Use undiluted 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 CD117/c-kit/SCF R controls (R&D Systems, Catalog # QC23).

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 ProClin® 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 or heparin 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.*
Elevated serum protein levels may interfere with this assay.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

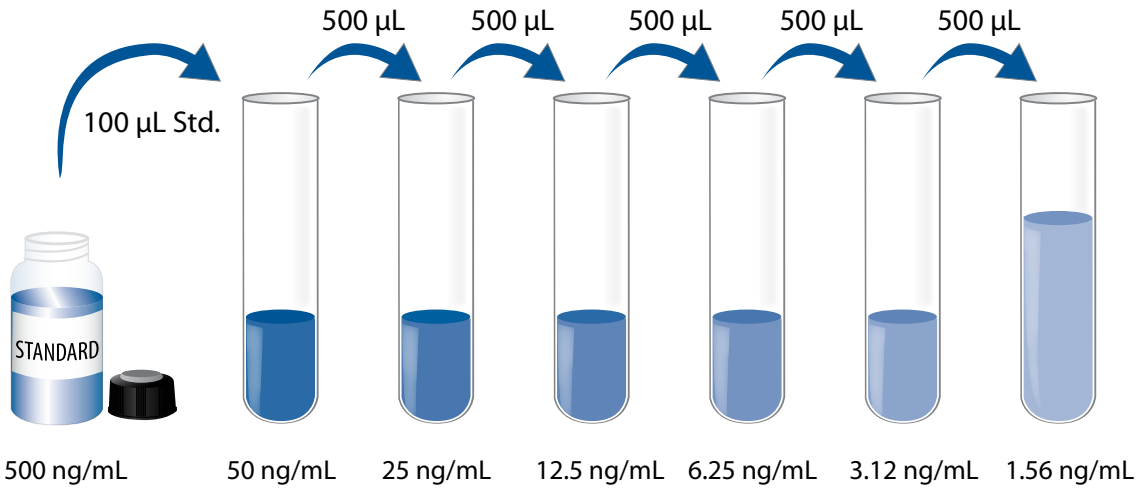
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 1:3) - **For cell culture supernate samples.** Add 5.0 mL of Calibrator Diluent RD5P Concentrate to 10 mL deionized or distilled water to prepare 15 mL of Calibrator Diluent RD5P (diluted 1:3).

Human SCF R Standard - Reconstitute the Human SCF R Standard with 1.0 mL of deionized or distilled water. This reconstitution produces a stock solution of 500 ng/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to making dilutions.

Pipette 900 µL of Calibrator Diluent RD5P (diluted 1:3) (*for cell culture supernate samples*) or Calibrator Diluent RD5P Concentrate (*for serum and plasma samples*) into the 50 ng/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 50 ng/mL standard serves as the high standard. The appropriate Calibrator Diluent serves as the zero standard (0 ng/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 RD1-19 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 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 SCF R 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.

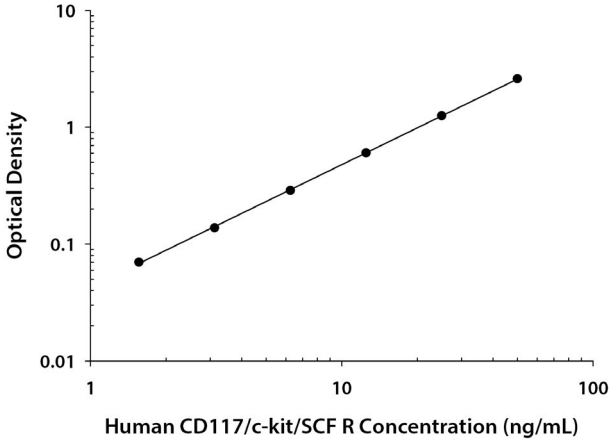
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on a log/log graph. The data may be linearized by plotting the log of the human CD117/c-kit/SCF R 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

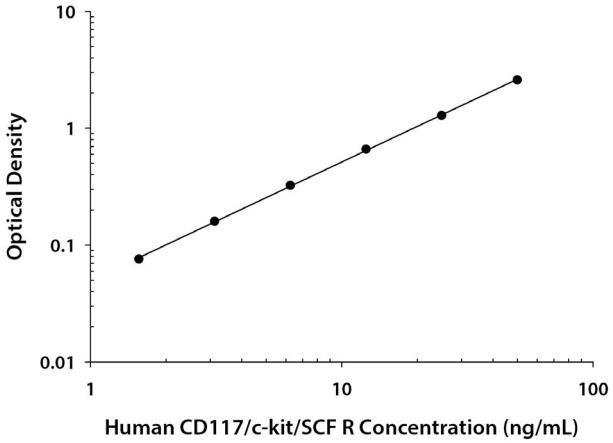
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.022 0.023	0.022	—
1.56	0.088 0.096	0.092	0.070
3.12	0.156 0.163	0.160	0.138
6.25	0.305 0.315	0.310	0.288
12.5	0.618 0.631	0.625	0.603
25	1.246 1.301	1.274	1.252
50	2.597 2.641	2.619	2.597

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.024 0.025	0.025	—
1.56	0.099 0.103	0.101	0.076
3.12	0.184 0.185	0.185	0.160
6.25	0.343 0.355	0.349	0.324
12.5	0.681 0.690	0.686	0.661
25	1.301 1.321	1.311	1.286
50	2.586 2.637	2.612	2.587

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 (ng/mL)	5.68	13.8	26.4	5.32	13.6	26.9
Standard deviation	0.228	0.390	0.707	0.481	0.509	1.21
CV (%)	4.0	2.8	2.7	9.0	3.7	4.5

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (ng/mL)	4.69	14.1	25.7	5.90	15.1	28.7
Standard deviation	0.298	0.359	0.562	0.555	0.688	1.76
CV (%)	6.4	2.5	2.2	9.4	4.6	6.1

RECOVERY

The recovery of human CD117/c-kit/SCF R spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	103	97-110%
Serum (n=4)	93	86-115%
EDTA plasma (n=4)	91	84-106%
Heparin plasma (n=4)	92	87-102%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human CD117/c-kit/SCF R 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	111	106	105	109
	Range (%)	106-113	103-109	98-109	106-111
1:4	Average % of Expected	111	110	108	107
	Range (%)	109-113	106-112	103-112	100-112
1:8	Average % of Expected	108	111	105	112
	Range (%)	105-110	108-114	98-112	108-116
1:16	Average % of Expected	108	107	102	101
	Range (%)	102-113	102-111	93-109	98-109

SENSITIVITY

Ninety assays were evaluated and the minimum detectable dose (MDD) of human CD117/c-kit/SCF R ranged from 0.057-0.339 ng/mL. The mean MDD was 0.165 ng/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 *Sf* 21-expressed recombinant human CD117/c-kit produced at R&D Systems.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human CD117/c-kit/SCF R in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Serum (n=35)	20.6	11.9-39.8	4.95
EDTA Plasma (n=35)	19.4	11.7-36.2	4.57
Heparin Plasma (n=35)	19.2	11.0-37.0	4.79

Cell Culture Supernates - Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% fetal calf 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 supernate were removed and assayed for levels of natural human CD117/c-kit/SCF R. No detectable levels were observed.

SPECIFICITY

This assay recognizes natural and recombinant human CD117/c-kit/SCF R.

The factors listed below were prepared at 500 ng/mL in Calibrator Diluent and assayed for cross-reactivity. Preparations of the following factors at 500 ng/mL in a mid-range recombinant human CD117/c-kit control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Flt-3 Ligand
M-CSF R
PDGF Rα
PDGF Rβ
SCF
VEGF R1
VEGF R2
VEGF R3

Other recombinants:

canine SCF
feline SCF
mouse SCF

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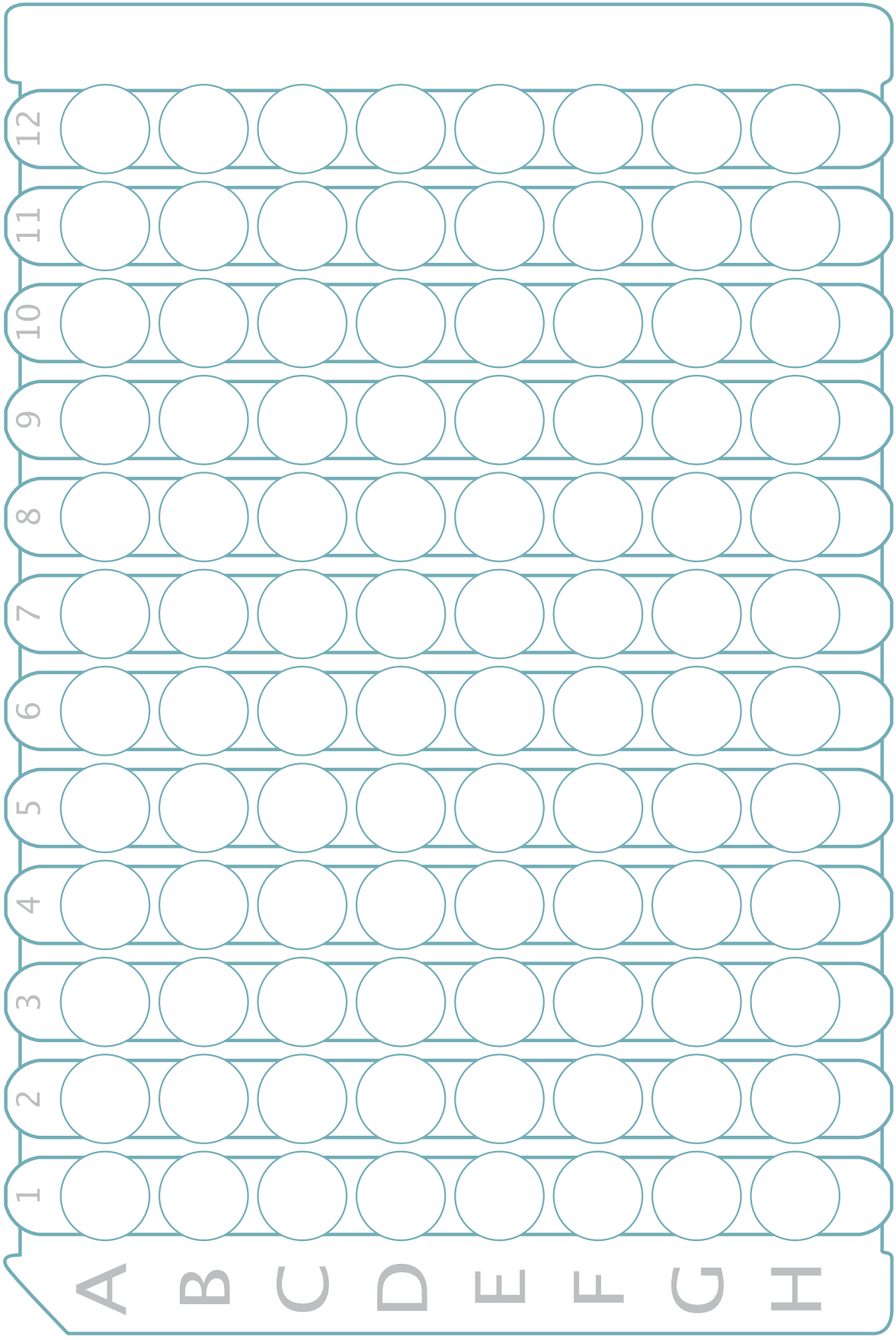
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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