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R&D SYSTEMS

Quantikine® ELISA

Human CXCL13/BLC/BCA-1 Immunoassay

Catalog Number DCX130

For the quantitative determination of human B-Lymphocyte Chemoattractant/B Cell-Attracting Chemokine 1 (BLC/BCA-1) concentrations in cell culture supernates, serum, plasma, and saliva.

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

B-Lymphocyte Chemoattractant (BLC), also known as B Cell-Attracting chemokine 1 (BCA-1), or CXCL13, is a member of the CXC subtype of the chemokine superfamily. BLC is critical for secondary lymphoid tissue development and navigation of lymphocytes within the microcompartments of these tissues. The gene encodes a putative protein of 109 amino acids (aa), including a 21 aa leader peptide (1, 2). At the aa level, human BLC exhibits 64% similarity to its mouse counterpart, and the gene has been mapped to chromosome segment 4q21 (1). Within the human BLC protein sequence, an arginine residue separates the first two of four conserved cysteine residues that are characteristic of CXC chemokines (1, 2).

BLC is a pertussis toxin-sensitive chemoattractant for B cells *in vitro* (1, 2). It is constitutively expressed in the B cell follicles of secondary lymphoid organs (1), and expression of BLC in these structures is dependent upon the activity of lymphotoxin α/β (3). BLC is also expressed in the pleural and peritoneal cavities (4), and in ectopic lymphoid follicles found within the synovium of patients with rheumatoid arthritis (5).

The primary BLC receptor is the 7-transmembrane G-protein coupled receptor, CXCR5, also known as Burkitt's lymphoma receptor 1 (BLR-1) (6). Cells that express CXCR5 and respond to BLC include B cells (1, 2, 4), follicular B helper T (T_{FH}) cells (7, 8), osteoblasts (9), podocytes (10), and a subset of skin-derived dendritic cells (11). In CXCR5-transfected HEK cells, BLC can stimulate elevations of Ca^{2+} and pertussis toxin-sensitive activation of MAP kinase signaling cascades (1, 2, 12). CXCR3, a known receptor for IFN-γ-inducible protein (IP-10) (13), monokine induced by IFN-γ (MIG) (13), and interferon-inducible T cell alpha chemoattractant (I-TAC) (14), is also activated *in vitro* by BLC (15).

$CXCL13^{-/-}$ and $CXCR5^{-/-}$ knockout mice exhibit similar abnormalities including deficiencies in the development of most peripheral lymphoid organs. Both knockouts have decreased numbers of peripheral lymph nodes and Peyer's patches, and a disruption of polarized B and T cell microcompartments in spleen and Peyer's patches (16, 17). Furthermore, $CXCR5^{-/-}$ B cell entry into Peyer's patches (homing) is impaired (18). BLC likely plays a complex role in antigen-induced movement of B cells within secondary lymphoid tissues. After antigen binding, B cells move from the follicle to the boundary of the T cell zone where they interact with helper T cells (19). Movement of B cells to the boundary of B and T cell zones is dependent upon the activity of CCR7, a receptor for the T cell zone chemokines MIP-3β/CCL19 and 6Ckine/CCL21 (20, 21). B cell translocation to the B/T cell boundary is inhibited by over-expression of CXCR5 (22). B1 cells, in contrast to conventional circulating B cells (B2 cells), home to the peritoneal and pleural cavities. This activity is inhibited in $CXCL13^{-/-}$ mice and is accompanied by a marked reduction in antibody responses to peritoneal antigens (4).

T_{FH} cells, a $CD4^+$ peripheral blood and tonsillar memory T cell subset, also express CXCR5 and migrate in response to BLC (8). Tonsillar T_{FH} cells cultured with tonsillar B cells stimulate IgG and IgA antibody production (7). *In vivo*, BLC co-localizes with T_{FH} cells in B cell zones of secondary lymphoid tissues suggesting a potential role in the regulation of humoral immunity (7, 8, 23).

The Quantikine® Human CXCL13/BLC/BCA-1 Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human CXCL13/BLC/BCA-1 in cell culture supernates, serum, plasma, and saliva. It contains *E. coli*-expressed recombinant human BLC/BCA-1 and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human BLC/BCA-1 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 BLC/BCA-1.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human BLC/BCA-1 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any BLC/BCA-1 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human BLC/BCA-1 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 BLC/BCA-1 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, further dilute the samples with 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 BLC/BCA-1 Microplate	892424	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human BLC/BCA-1.	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 BLC/BCA-1 Conjugate	892425	21 mL of a monoclonal antibody specific for human BLC/BCA-1 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human BLC/BCA-1 Standard	892426	Recombinant human BLC/BCA-1 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1S	895137	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD6-41	895840	21 mL of a buffered protein base with preservatives.	
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.
- Collection device for saliva samples that has no protein binding or filtering capabilities such as a Salivette® or equivalent.
- Test tubes for dilution of standards and samples.
- Human BLC/BCA-1 Controls (R&D Systems®, Catalog # QC48).

PRECAUTIONS

Human BLC/BCA-1 is detectable in saliva. Take precautionary measures to prevent contamination of kit reagents while running this assay.

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 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.*

Saliva - Collect saliva using a collection device such as a Salivette® or equivalent. Assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Note: *Saliva collector must not have any protein binding or filtering capabilities.*

SAMPLE PREPARATION

Saliva samples require a 2-fold dilution. A suggested 2-fold dilution is 100 µL of sample + 100 µL of Calibrator Diluent RD6-41.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

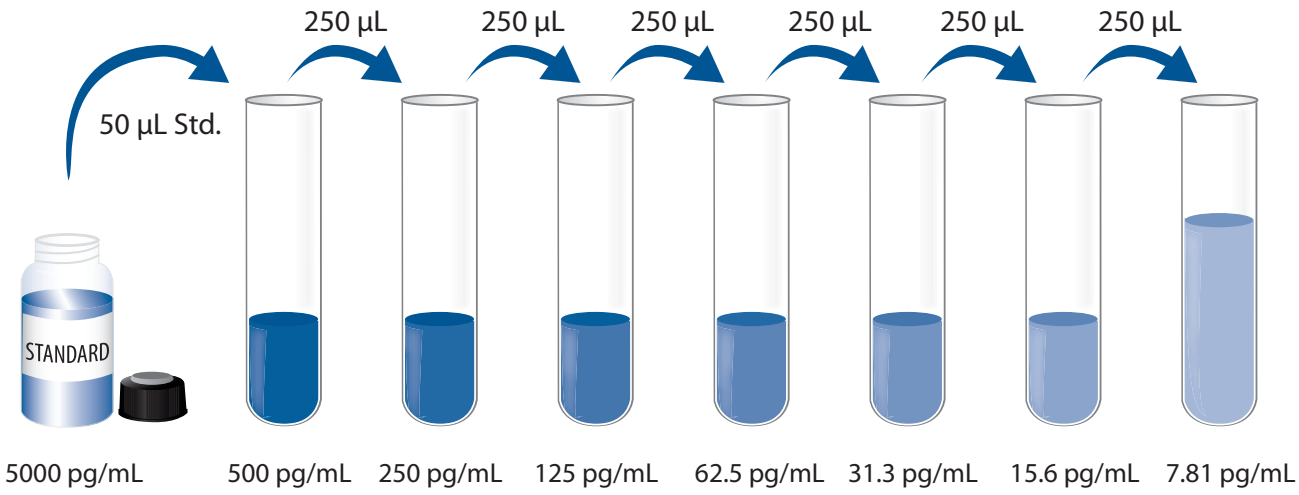
Note: *BLC/BCA-1 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 BLC/BCA-1 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human BLC/BCA-1 Standard with deionized or distilled water. This reconstitution produces a stock solution of 5,000 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 450 µL of Calibrator Diluent RD6-41 into the 500 pg/mL tube. Pipette 250 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. Calibrator Diluent RD6-41 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 samples, controls, and standards be assayed in duplicate.

Note: *BLC/BCA-1 is found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

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 RD1S 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 BLC/BCA-1 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.

*Samples may require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

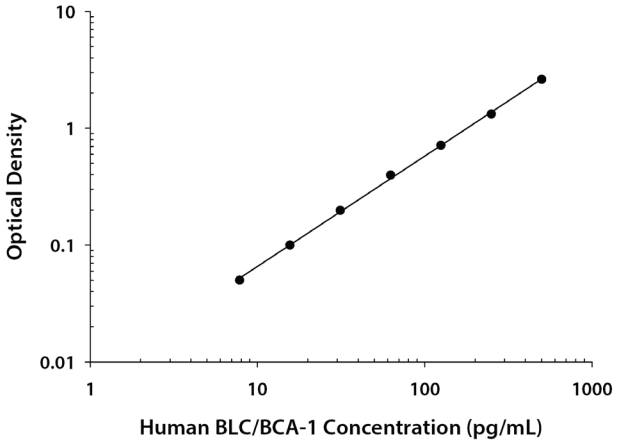
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

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 BLC/BCA-1 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

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.058 0.061	0.060	—
7.81	0.107 0.113	0.110	0.050
15.6	0.159 0.161	0.160	0.100
31.3	0.257 0.259	0.258	0.198
62.5	0.454 0.459	0.457	0.397
125	0.758 0.789	0.774	0.714
250	1.297 1.464	1.381	1.321
500	2.655 2.700	2.678	2.618

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.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	40	40	40
Mean (pg/mL)	50.2	136	294	54.0	138	285
Standard deviation	2.2	4.5	8.0	5.1	13.2	24.8
CV (%)	4.4	3.3	2.7	9.4	9.6	8.7

RECOVERY

The recovery of human BLC/BCA-1 spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	98	87-110%
Serum (n=4)	99	86-108%
EDTA plasma (n=4)	99	92-107%
Heparin plasma (n=4)	101	92-115%

LINEARITY

To assess the linearity of the assay, samples containing and/or spiked with high concentrations of human BLC/BCA-1 were diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=3)	Cell culture media (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva* (n=4)
1:2	Average % of Expected	104	101	96	97	95	105
	Range (%)	102-108	99-105	93-101	94-100	92-100	103-109
1:4	Average % of Expected	107	104	93	100	96	101
	Range (%)	103-112	101-106	91-96	91-108	86-103	94-109
1:8	Average % of Expected	103	102	94	98	101	92
	Range (%)	98-106	99-105	86-100	88-106	98-105	92-92
1:16	Average % of Expected	97	102	94	96	108	———
	Range (%)	94-99	98-110	93-96	94-99	108-108	———

*Samples were diluted prior to assay as directed in the Sample Preparation section.

SENSITIVITY

Fifty-one assays were evaluated and the minimum detectable dose (MDD) of human BLC/BCA-1 ranged from 0.43-3.97 pg/mL. The mean MDD was 1.64 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 *E. coli*-expressed recombinant human BLC/BCA-1 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Saliva - Samples from apparently healthy volunteers were evaluated for the presence of human BLC/BCA-1 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=35)	81.9	39.4-252	43.5
EDTA plasma (n=35)	62.5	29.4-246	42.7
Heparin plasma (n=35)	63.5	21.7-240	44.1

*Twenty-five additional serum samples were tested and values of 10.6 pg/mL, 379 pg/mL, and 638 pg/mL were observed in three samples. The remaining samples were consistent with the range listed above.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Saliva (n=10)	67.4	70	ND-284

ND=Non-detectable

Cell Culture Supernates:

Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in RPMI supplemented with 10% 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 supernate were removed and assayed for levels of human BLC/BCA-1.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND
Stimulated	11	640

ND=Non-detectable

THP-1 human acute monocytic leukemia cells were cultured in RPMI supplemented with 10% fetal bovine serum, stimulated with 1.0 µg/mL of recombinant human IFN-γ for eight hours, and then stimulated with 1.0 µg/mL of LPS overnight. An aliquot was removed, assayed for detectable human BLC/BCA-1, and measured 15,922 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human BLC/BCA-1.

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 BLC/BCA-1 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

CXCL16
ENA-78
GCP-2
GROα
GROβ
GROγ
IL-8
IL-8, endothelial cell-derived
IP-10
I-TAC
MIG
NAP-2
SDF-1α
SDF-1β

Recombinant mouse:

BLC/BCA-1
GCP-2
I-TAC
KC
MIG
SDF-1α

Natural proteins:

porcine IL-8

Recombinant mouse IP-10/CRG-2 cross-reacts approximately 0.036% in this assay.

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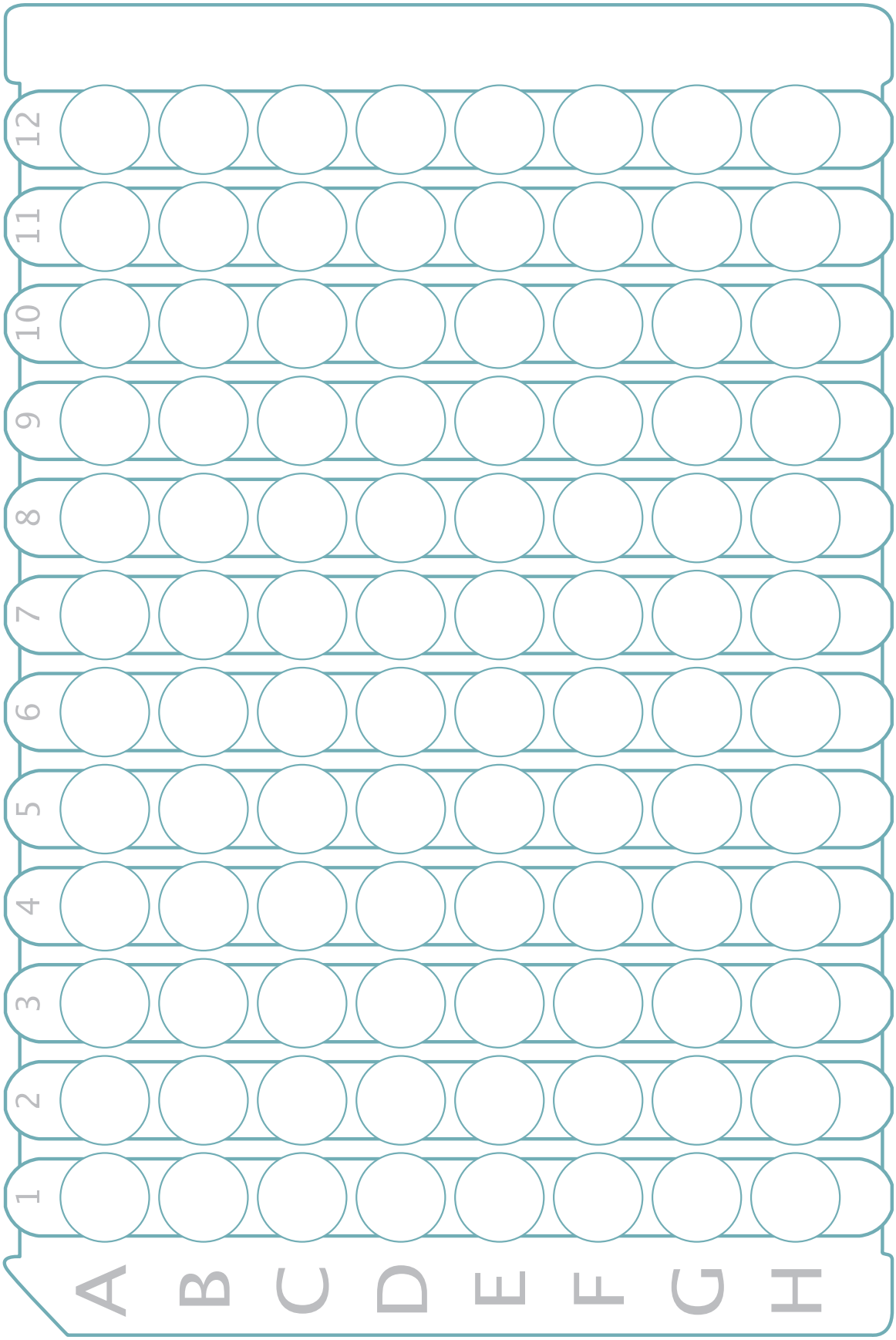
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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