Quantikine® ELISA

Human Galectin-3BP/MAC-2BP Immunoassay

Catalog Number DGBP30B

For the quantitative determination of human Galectin-3 Binding Protein (Galectin-3BP) concentrations in cell culture supernates, serum, plasma, saliva, and urine.

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

Galectin-3 Binding Protein (Galectin-3BP), also known as MAC-2 Binding Protein/M2BP, 90K, Tumor-associated Antigen 90K, and basement Membrane Autoantigen p105, is an 80-100 kDa secreted Group A member of the Scavenger Receptor Cysteine-rich (SPCR) domain superfamily of molecules (1-3). It is a variably glycosylated, cysteine-rich, monomeric protein that plays an apparent role in both cancer biology and inflammatory disease processes (1, 5-7). Human Galectin-3BP is synthesized as a 585 amino acid (aa) precursor that contains an 18 aa signal sequence plus a 567 aa mature segment (8, 9). The mature region contains an N-terminal A-type SRCR region (aa 24-124), followed by a BTB domain (aa 153-221), one IVR/BACK (BTB and Kelch) domain (aa 261-358), and a nondescript C-terminal tail (1, 3, 9-11). Upon secretion, Galectin-3BP is rarely, if ever, found in monomeric form. Instead, it is found to exist as either a noncovalent homodimer (in parallel), or a noncovalent hexamer composed of six homodimers that create a 30-40 nm ring structure (10-12). Either the C-terminus or SRCR domain plausibly could be ligand-binding structures, while domains 2 through 4 all likely contribute to multimerization (10, 11, 13). Galectin-3BP will potentially undergo extracellular proteolytic cleavage after Arg435, generating a 70-72 kDa and 27 kDa isoform (9, 14, 15). It is suggested that the 70 kDa isoform is bound to the cell-surface, while the 95 kDa isoform likely forms multimers (13). Mature human Galectin-3BP shares 69% aa sequence identity with a purported mouse ortholog termed CyCAP/MAMA (16, 17). Human Galectin-3BP is secreted by multiple normal cell types, including bone marrow stromal and synovial fibroblasts (4, 15), prostatic epithelium (13), macrophages and pancreatic islet cells (18), and skeletal muscle (12). It is also secreted by multiple tumor cell types, including those derived from breast duct epithelium (19), neuroblastoma (15), melanoma (20), and pancreatic duct epithelium (18). Rodent CyCAP is also secreted by numerous cells, including B cells, neurons, astrocytes, breast epithelium, and microglia (16, 21). At issue is whether these rodent results can be extrapolated to human Galectin-3BP. Rodent CyCAP binds cyclophilin C while human Galectin-3BP does not; conversely, human Galectin-3BP binds MAC-2/Galectin-3 while CyCAP does not (22). Thus, it is not clear if mouse CyCAP is a true functional ortholog of human Galectin-3BP.

Galectin-3BP has multiple functions associated with it. As noted, it influences both cancer biology and immune responses. With respect to cancer biology, it has been suggested that tumor-expressed Galectin-3BP may interact with the dendritic cell (DC) DC-SIGN, blocking the maturation of immature monocyte-derived DCs and creating a tolerogenic environment (14). In addition, Galectin-3BP is also reported to induce cell aggregation through binding to its ring structures, promote cell attachment to endothelium, and induce the cleavage of tumor cell membrane PTPκ, thus blocking cell adhesion (5, 23, 24). In total, these phenomena have the potential to contribute to the metastatic process. Conversely, Galectin-3BP has also been found to promote both NK and $CD8^+$ T cell lysis of tumor cells, augmenting their antigenicity partly through an upregulation of MHC Class I molecules (25, 26). With respect to the inflammatory process, Galectin-3BP appears to promote a Th1-type environment by inducing NK cell expansion, promoting IL-2 and TNF-α secretion, and inhibiting monocyte production of IL-4, IL-5, and IL-13 (1, 8, 15, 25). Ligands identified for Galectin-3BP include Galectin-1, -3 and -7 (2, 27), E-Selectin (23), Tem1 (13), DC-SIGN (14), plus fibronectin, nidogen-1, and collagens IV, V and VI (10, 12).

The Quantikine Human Galectin-3BP/MAC-2BP Immunoassay is a 4.5 hour solid-phase ELISA designed to measure human Galectin-3BP in cell culture supernates, serum, plasma, saliva, and urine. It contains NS0-expressed recombinant human Galectin-3BP and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human Galectin-3BP 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 Galectin-3BP.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Galectin-3BP has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Galectin-3BP present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Galectin-3BP 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 Galectin-3BP 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 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 Galectin-3BP Microplate	894527	One 96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Galectin-3BP.	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 Galectin-3BP Standard	894529	2 vials (50 ng/vial) of recombinant human Galectin-3BP in a buffered protein base with preservatives; lyophilized.	Use a new Standard for each assay. Discard after use.
Human Galectin-3BP Conjugate	894528	21 mL of a polyclonal antibody specific for human Galectin-3BP conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
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 1:10 in this assay.*	
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.
- 50 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human Galectin-3BP Controls (optional; R&D Systems, Catalog # QC95).

PRECAUTIONS

Galectin-3BP 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. 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.*

Saliva - Collect saliva in a tube and centrifuge for 5 minutes at 10,000 x g. Collect the aqueous layer, and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

Urine - Aseptically collect the first urine of the day (mid-stream), voided directly into a sterile container. Centrifuge to remove particulate matter, and assay immediately or aliquot and store at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

SAMPLE PREPARATION

Serum and plasma samples require a 500-fold dilution. A suggested 500-fold dilution can be achieved by adding 10 µL of sample to 490 µL of Calibrator Diluent RD5P (diluted 1:10)*. Complete the 500-fold dilution by adding 20 µL of the diluted sample to 180 µL Calibrator Diluent RD5P (diluted 1:10).

Saliva samples require at least a 50-fold dilution. A suggested 50-fold dilution is 10 µL of sample + 490 µL of Calibrator Diluent RD5P (diluted 1:10).

Urine samples require a 30-fold dilution. A suggested 30-fold dilution is 10 µL of sample + 290 µL of Calibrator Diluent RD5P (diluted 1:10).

*See Reagent Preparation section.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *High concentrations of Galectin-3BP are found in saliva. It is recommended that a face mask and gloves be used to protect kit reagents from contamination.*

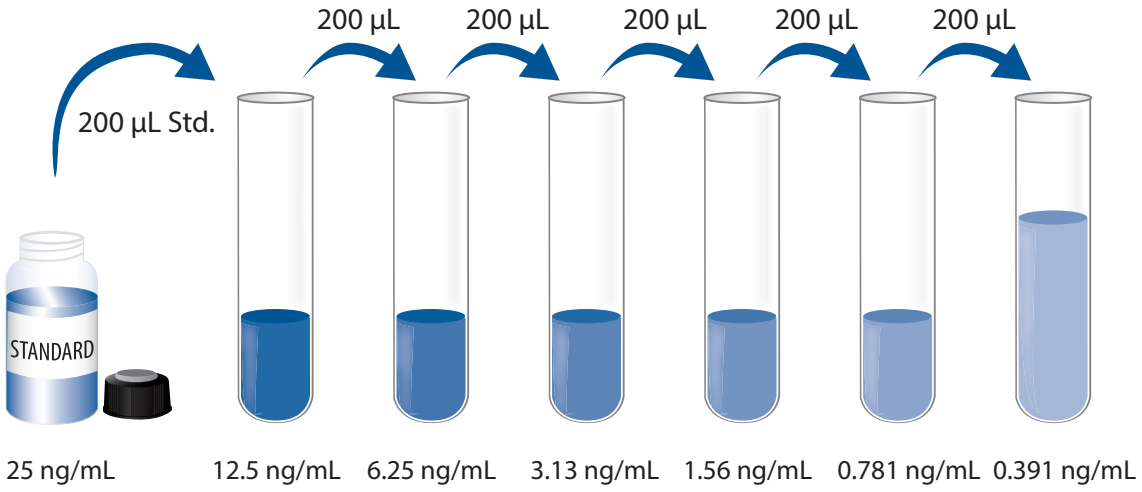
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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.

Calibrator Diluent RD5P (diluted 1:10) - Add 5 mL of Calibrator Diluent RD5P Concentrate to 45 mL of deionized or distilled water to prepare 50 mL of Calibrator Diluent RD5P (diluted 1:10).

Human Galectin-3BP Standard - Reconstitute the Human Galectin-3BP Standard with 2.0 mL of Calibrator Diluent RD5P (diluted 1:10). This reconstitution produces a stock solution of 25 ng/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 200 µL of Calibrator Diluent RD5P (diluted 1:10) into each tube. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The undiluted Galectin-3BP Standard (25 ng/mL) serves as the high standard. Calibrator Diluent RD5P (diluted 1:10) 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 samples, controls, and standards be assayed in duplicate.

Note: *High concentrations of Galectin-3BP are 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 50 µL of Assay Diluent RD1-19 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 on a horizontal orbital microplate shaker (0.12" orbit) set at 500 ± 50 rpm. A plate layout is provided for a record of 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 Galectin-3BP Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
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 **on the benchtop. 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 the Sample Preparation section.

CALCULATION OF RESULTS

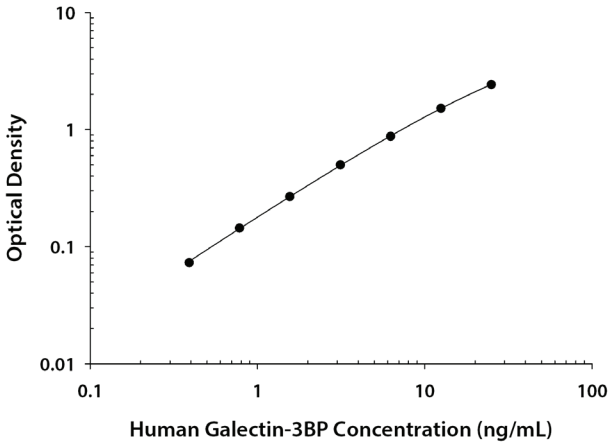
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) 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 the graph. The data may be linearized by plotting the log of the human Galectin-3BP concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

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.



(ng/mL)	O.D.	Average	Corrected
0	0.014 0.014	0.014	—
0.391	0.086 0.088	0.087	0.073
0.781	0.155 0.160	0.158	0.144
1.56	0.277 0.285	0.281	0.267
3.13	0.507 0.519	0.513	0.499
6.25	0.887 0.891	0.889	0.875
12.5	1.495 1.567	1.531	1.517
25	2.425 2.451	2.438	2.424

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 thirty-two 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	32	32	32
Mean (ng/mL)	0.743	2.94	8.27	0.778	3.20	9.10
Standard deviation	0.027	0.080	0.244	0.078	0.225	0.434
CV (%)	3.6	2.7	3.0	10.0	7.0	4.8

RECOVERY

The recovery of human Galectin-3BP spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=8)	99	95-101%
Saliva* (n=3)	91	82-101%
Urine* (n=4)	104	98-111%

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

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human Galectin-3BP were diluted with Calibrator Diluent to produce samples with values within the dynamic range of the assay. Samples were diluted prior to assay.

		Cell culture supernates (n=4)	Serum (n=4)	EDTA plasma (n=4)	Heparin plasma (n=4)	Saliva (n=4)	Urine (n=4)
1:2	Average % of Expected	103	99	100	104	106	98
	Range (%)	98-108	97-103	97-104	100-107	97-115	93-102
1:4	Average % of Expected	102	100	102	104	102	97
	Range (%)	96-109	92-106	99-105	100-110	91-114	94-99
1:8	Average % of Expected	101	101	106	107	99	94
	Range (%)	93-110	87-114	101-109	100-112	84-110	———
1:16	Average % of Expected	107	105	108	106	98	———
	Range (%)	95-117	94-115	100-115	100-113	88-110	———

SENSITIVITY

Sixty-five assays were evaluated and the minimum detectable dose (MDD) of human Galectin-3BP ranged from 0.006-0.161 ng/mL. The mean MDD was 0.022 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 NS0-expressed recombinant human Galectin-3BP manufactured at R&D Systems.

SAMPLE VALUES

Serum/Plasma/Saliva/Urine - Samples from apparently healthy volunteers were evaluated for the presence of human Galectin-3BP in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean (µg/mL)	Range (µg/mL)	Standard Deviation (µg/mL)
Serum (n=35)	5.4	1.3-18.4	3.3
EDTA plasma (n=35)	4.9	1.1-18.1	3.1
Heparin plasma (n=35)	5.1	1.1-17.1	3.0

Sample Type	Mean (ng/mL)	Range (ng/mL)	Standard Deviation (ng/mL)
Saliva (n=7)	328	28.7-720	230
Urine (n=11)	162	42.9-452	114

Cell Culture Supernates:
HepG2 human hepatocellular carcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Galectin-3BP, and measured 627 ng/mL.

OVCAR-3 human ovarian carcinoma cells were cultured in RPMI supplemented with 20% fetal bovine serum, 10 µg/mL bovine insulin, 10 mM HEPES, 1 mM sodium pyruvate, 4.5 g/L glucose, and 1.5 g/L sodium bicarbonate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Galectin-3BP, and measured 263 ng/mL.

SK-Mel-28 human malignant melanoma cells were cultured in MEM NEAA supplemented with 10% fetal bovine serum, 1 mM sodium pyruvate, 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin sulfate until confluent. An aliquot of the cell culture supernate was removed, assayed for human Galectin-3BP, and measured 119 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Galectin-3BP.

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 Galectin-3BP control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

DC-SIGN
E-Selectin
Fibronectin-1
Galectin-1
Galectin-2
Galectin-3
Galectin-4
Galectin-7
Galectin-8
Galectin-10
Galectin-14
Integrin-β1
Nidogen-1
Nidogen-2

Natural proteins:

bovine Collagen-1
human Fibronectin

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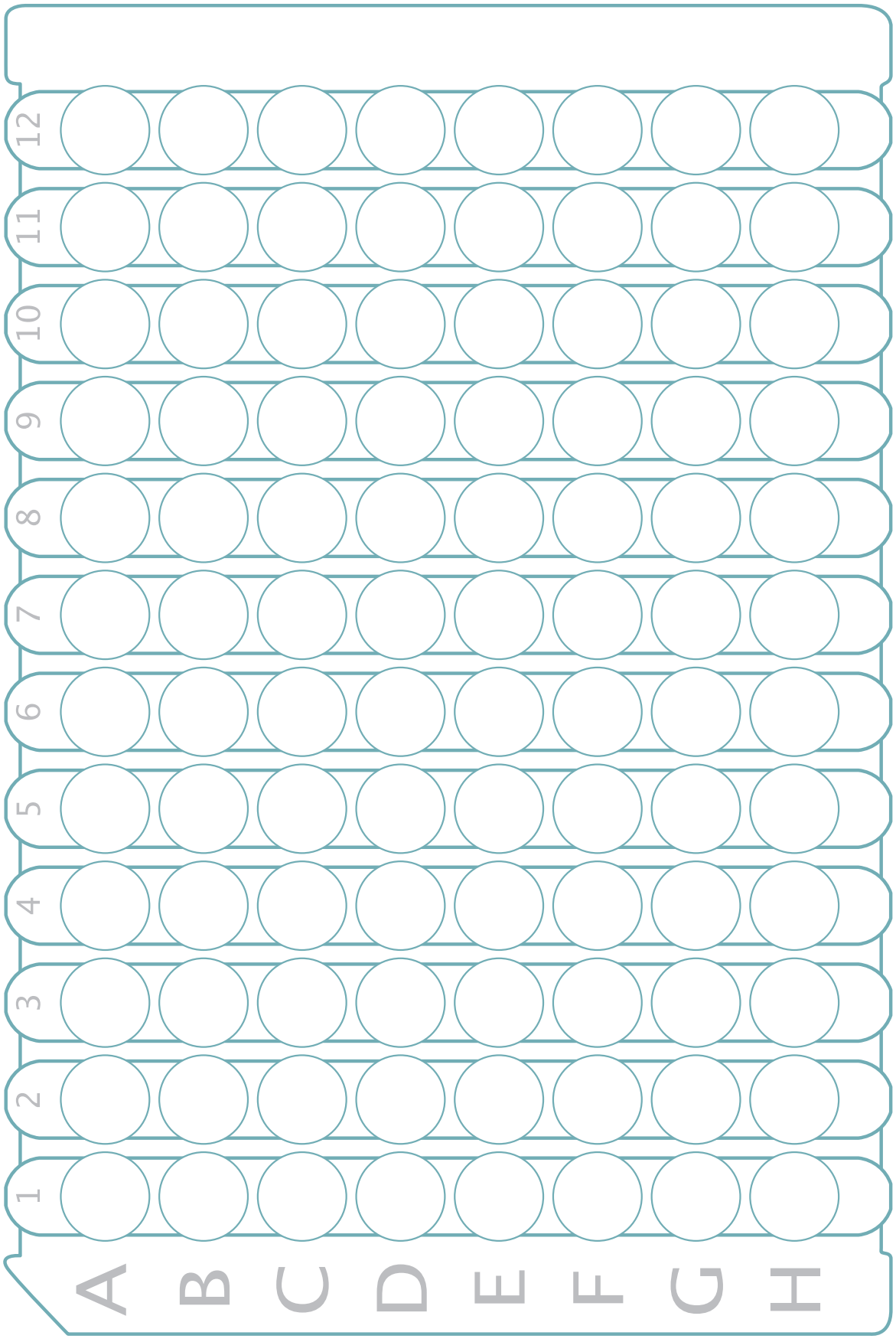
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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