Quantikine® ELISA

Human Galectin-3 Immunoassay

Catalog Number DGAL30
SGAL30
PDGAL30

For the quantitative determination of human Galectin-3 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

Galectins are a family of carbohydrate-binding proteins with specificity for N-acetyl-lactosamine (LacNAc)-containing glycoproteins (1). At least 14 mammalian galectins share structural similarities in their carbohydrate recognition domains (CRD). Most fall within two groups called prototype (one CRD) or tandem-repeat (two CRDs). Galectin-3, also known as Mac-2 and previously as L29, CBP35, or eBP, is the only known Galectin with one CRD and a unique N-terminus. It is often termed a chimeric galectin (1-4). Galectins lack classical signal peptides but are present and active both within and outside of the cell. They are involved in cell adhesion, migration, survival, and apoptosis and are often up- or down-regulated in cancer (1). Human and mouse Galectin-3 share approximately 86% amino acid (aa) sequence identity. The 250 aa, 29-36 kDa human Galectin-3 is widely expressed in various cells, including macrophages, activated T cells, osteoclasts, epithelial cells, tumor cells, and fibroblasts (3, 5). Galectin-3 expression is up-regulated in macrophages as compared to monocytes or dendritic cells (6). Increased serum Galectin-3 has been noted in rheumatoid arthritis (also in synovial fluid), Behcet's disease, and a variety of cancers, especially when they are metastatic (5, 7-10). Cleavage of Galectin-3 in tumors is highly indicative of matrix metalloproteinase activity (11).

Galectin-3 is highly pleotrophic in function and has numerous intracellular and extracellular binding partners (2). The Galectin-3 CRD recognizes terminal, unsialated LacNAc structures that are present on approximately 20% of all serum proteins and many extracellular matrix proteins, while its eight tandem repeats within the unique N-terminal domain participate in protein-protein interactions (2-4, 12, 13). Nuclear Galectin-3 can alter gene expression, while in the cytosol, it can inhibit apoptosis and participate in exocytosis, caveolin-mediated endocytosis, and macrophage clearance of apoptotic cells (2, 3, 14). Extracellular Galectin-3 is involved in innate immunity, binding carbohydrates on specific pathogens such as *Candida albicans* and *Streptococcus pneumoniae*, and acting as an opsonin (15-17). It is chemotactic for macrophages and induces innate immune responses in neutrophils (1, 6, 18, 19). It cross-links CD98 to activate macrophages by the alternate pathway (20). Although many of its extracellular functions are pro-inflammatory and pro-apoptotic, it can be anti-inflammatory by down-regulating macrophage responses to bacterial lipopolysaccharides (3, 13, 21). Galectin-3 binding to endothelial cells stimulates angiogenesis, while fibroblast binding can promote fibrosis (22, 23). Some of these activities involve its adhesion to or regulation of certain integrins (2, 13, 24, 25). Galectin-3 is monomeric, but it can form pentamers when the CRD is engaged by a carbohydrate, creating lattice structures on the cell surface (25-28). These lattices are involved in regulating focal adhesion dynamics and CD45-mediated control of T cell receptor signaling (29, 30). It participates in lipid rafts and influences receptor dimerization or clustering, potentially regulating signaling, adhesion, and receptor endocytosis (31, 32).

The Quantikine® Human Galectin-3 Immunoassay is a 4.5 hour solid phase ELISA designed to measure human Galectin-3 levels in cell culture supernates, serum, and plasma. It contains *E. coli*-expressed recombinant human Galectin-3 and antibodies raised against the recombinant protein. Results obtained for naturally occurring human Galectin-3 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 Galectin-3.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Galectin-3 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Galectin-3 present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Galectin-3 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-3 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 #	CATALOG # DGAL30	CATALOG # SGAL30	DESCRIPTION	STORAGE OF OPENED, DILUTED, OR RECONSTITUTED MATERIAL
Human Galectin-3 Microplate	893751	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Galectin-3.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of zip-seal. May be stored for up to 1 month at 2-8 °C.*
Calibrator Diluent RD6X	895152	1 vial	6 vials	21 mL/vial of animal serum with preservatives. *For serum/plasma samples. Use diluted 1:5 in this assay.*	Discard after use. Use fresh for each assay.
Human Galectin-3 Conjugate	893752	1 vial	6 vials	21 mL/vial of a polyclonal antibody specific for human Galectin-3 conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Human Galectin-3 Standard	893753	1 vial	6 vials	Recombinant human Galectin-3 in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	
Assay Diluent RD1W	895117	1 vial	6 vials	11 mL/vial of a buffered protein base with preservatives.	
Calibrator Diluent RD5K	895119	1 vial	6 vials	21 mL/vial of a buffered protein base with preservatives. *For cell culture supernate samples.*	
Wash Buffer Concentrate	895003	1 vial	6 vials	21 mL/vial of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	1 vial	6 vials	12 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	1 vial	6 vials	6 mL/vial of 2 N sulfuric acid.	
Plate Sealers	N/A	4 strips	24 strips	Adhesive strips.	

* Provided this is within the expiration date of the kit.

DGAL30 contains sufficient materials to run an ELISA on one 96 well plate.
SGAL30 (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PDGAL30). PharmPaks contain sufficient materials to run ELISAs on 50 microplates. Specific vial counts of each component may vary. Refer to the literature accompanying your order for specific vial counts.

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.
- Test tubes for dilution of standards.
- Human Galectin-3 Controls (optional; R&D Systems®, Catalog # QC24).

PRECAUTIONS

Galectin-3 is found in saliva. Take necessary precautions to protect kit reagents (i.e. face mask and gloves).

Calibrator Diluent RD6X 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 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 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.*

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Note: *Galectin-3 is found in saliva. Take necessary precautions to protect kit reagents (i.e. face mask and gloves).*

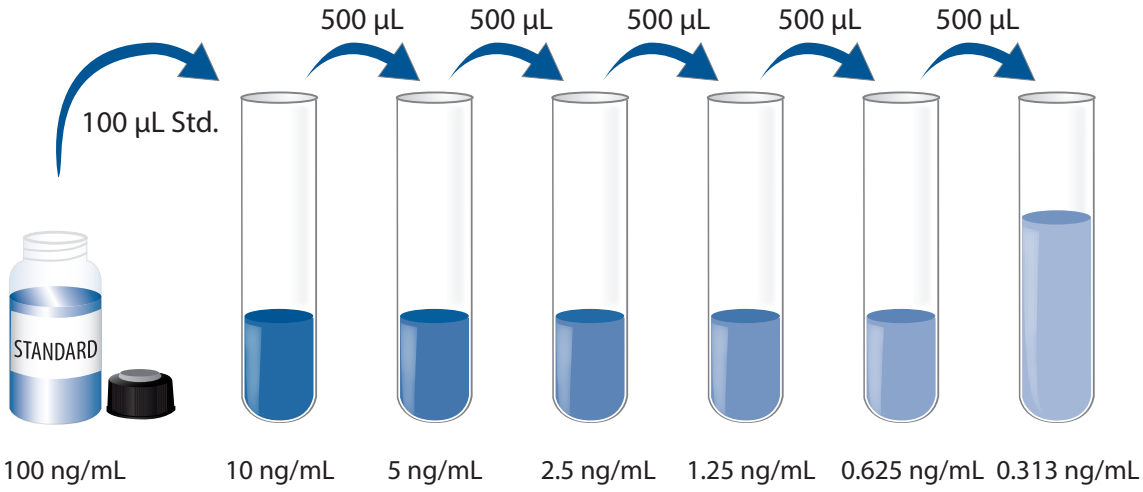
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD6X (diluted 1:5) - Add 1 mL of Calibrator Diluent RD6X to 4 mL deionized or distilled water to prepare 5 mL of Calibrator Diluent RD6X (diluted 1:5). **Prepare only as much needed to run the assay. Discard after use.**

Human Galectin-3 Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Galectin-3 Standard with deionized or distilled water. This reconstitution produces a stock solution of 100 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 RD5K (*for cell culture supernate samples*) or Calibrator Diluent RD6X (diluted 1:5) (*for serum/plasma samples*) into the 10 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 10 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.

Note: *Galectin-3 is found in saliva. Take necessary precautions to protect kit reagents (i.e. face mask and gloves).*

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 RD1W to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided and 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 Galectin-3 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 (O.D.).

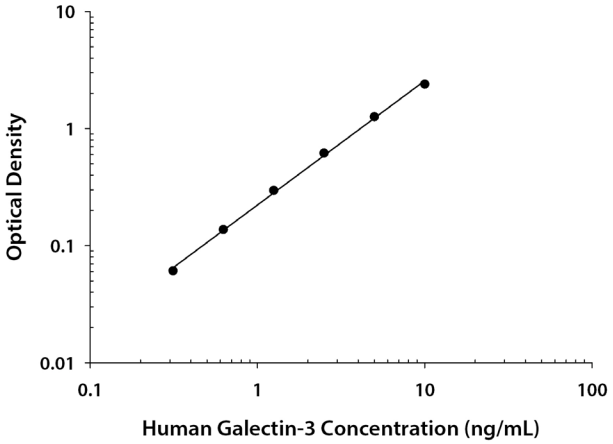
Create a standard curve by reducing the data using computer software capable of generating a log/log curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points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

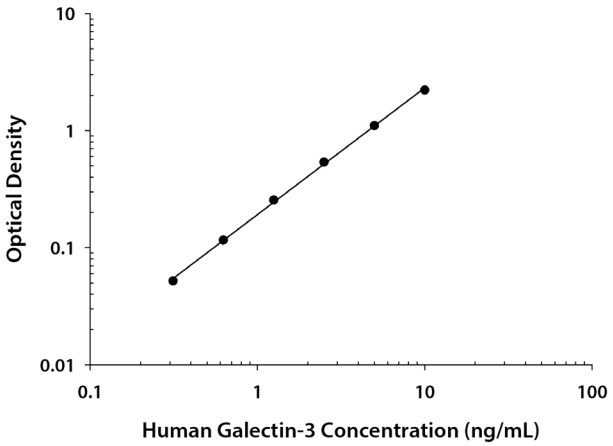
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.008 0.008	0.008	—
0.313	0.068 0.069	0.069	0.061
0.625	0.145 0.145	0.145	0.137
1.25	0.300 0.308	0.304	0.296
2.5	0.622 0.628	0.625	0.617
5.0	1.267 1.267	1.267	1.259
10	2.378 2.422	2.400	2.392

SERUM/PLASMA ASSAY



(ng/mL)	O.D.	Average	Corrected
0	0.010 0.010	0.010	—
0.313	0.062 0.062	0.062	0.052
0.625	0.125 0.127	0.126	0.116
1.25	0.262 0.267	0.265	0.255
2.5	0.542 0.551	0.547	0.537
5.0	1.095 1.122	1.109	1.099
10	2.170 2.281	2.226	2.216

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)	0.78	2.27	5.06	0.81	2.51	5.29
Standard deviation	0.03	0.10	0.15	0.07	0.20	0.36
CV (%)	3.8	4.4	3.0	8.6	8.0	6.8

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)	0.79	2.46	5.11	0.80	2.59	5.50
Standard deviation	0.03	0.09	0.18	0.05	0.16	0.32
CV (%)	3.8	3.7	3.5	6.3	6.2	5.8

RECOVERY

The recovery of human Galectin-3 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=4)	102	96-106%
Serum (n=4)	104	94-115%
EDTA plasma (n=4)	105	93-113%
Heparin plasma (n=4)	103	95-110%

LINEARITY

To assess linearity of the assay, samples were containing and/or spiked with high concentrations of human Galectin-3 in various matrices were 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	103	108	110	106
	Range (%)	100-106	103-116	106-113	105-108
1:4	Average % of Expected	102	105	103	102
	Range (%)	99-104	101-112	100-106	97-106
1:8	Average % of Expected	100	101	100	96
	Range (%)	99-103	98-104	99-102	89-102
1:16	Average % of Expected	98	93	95	92
	Range (%)	93-104	88-97	87-100	86-101

SENSITIVITY

Eighty-seven assays were evaluated and the minimum detectable dose (MDD) of human Galectin-3 ranged from 0.003-0.085 ng/mL. The mean MDD was 0.016 ng/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 Galectin-3 produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma - Samples from apparently healthy volunteers were evaluated for the presence of human Galectin-3 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=36)	6.73	2.40-15.7	2.0
EDTA plasma (n=36)	6.44	2.03-15.5	2.1
Heparin plasma (n=36)	6.39	1.93-14.3	1.9

Cell Culture Supernates:
Human peripheral blood lymphocytes (PBLs) were cultured in DMEM supplemented with 5% fetal bovine serum, 5 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 of PHA for 1 and 6 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Galectin-3.

Condition	Day 1 (ng/mL)	Day 6 (ng/mL)
Unstimulated	0.542	0.557
Stimulated	1.50	5.56

COLO 205 human colorectal adenocarcinoma cells were cultured in RPMI and supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human Galectin-3, and measured 0.880 ng/mL.

HT-29 human colon adenocarcinoma cells were cultured in McCoy's 5a media supplemented with 10% fetal bovine serum and 2 mM L-glutamine. An aliquot of the cell culture supernate was removed, assayed for human Galectin-3, and measured 0.818 ng/mL.

SK-HEP-1 human liver adenocarcinoma cells were cultured in DMEM supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 1 mM sodium pyruvate. An aliquot of the cell culture supernate was removed, assayed for human Galectin-3, and measured 0.388 ng/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Galectin-3.

The factors listed below were prepared at 100 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 100 ng/mL in a mid-range Galectin-3 control were assayed for interference. No significant cross-reactivity or interference was observed.

Recombinant human:

Galectin-1
Galectin-2
Galectin-4
Galectin-7
Galectin-8
Galectin-9
Galectin-10
Galectin-14
MAC-2BP

Recombinant mouse:

Galectin-1
Galectin-2
Galectin-7
MAC-2BP

Recombinant mouse Galectin-3 cross-reacts approximately 58% in this assay.

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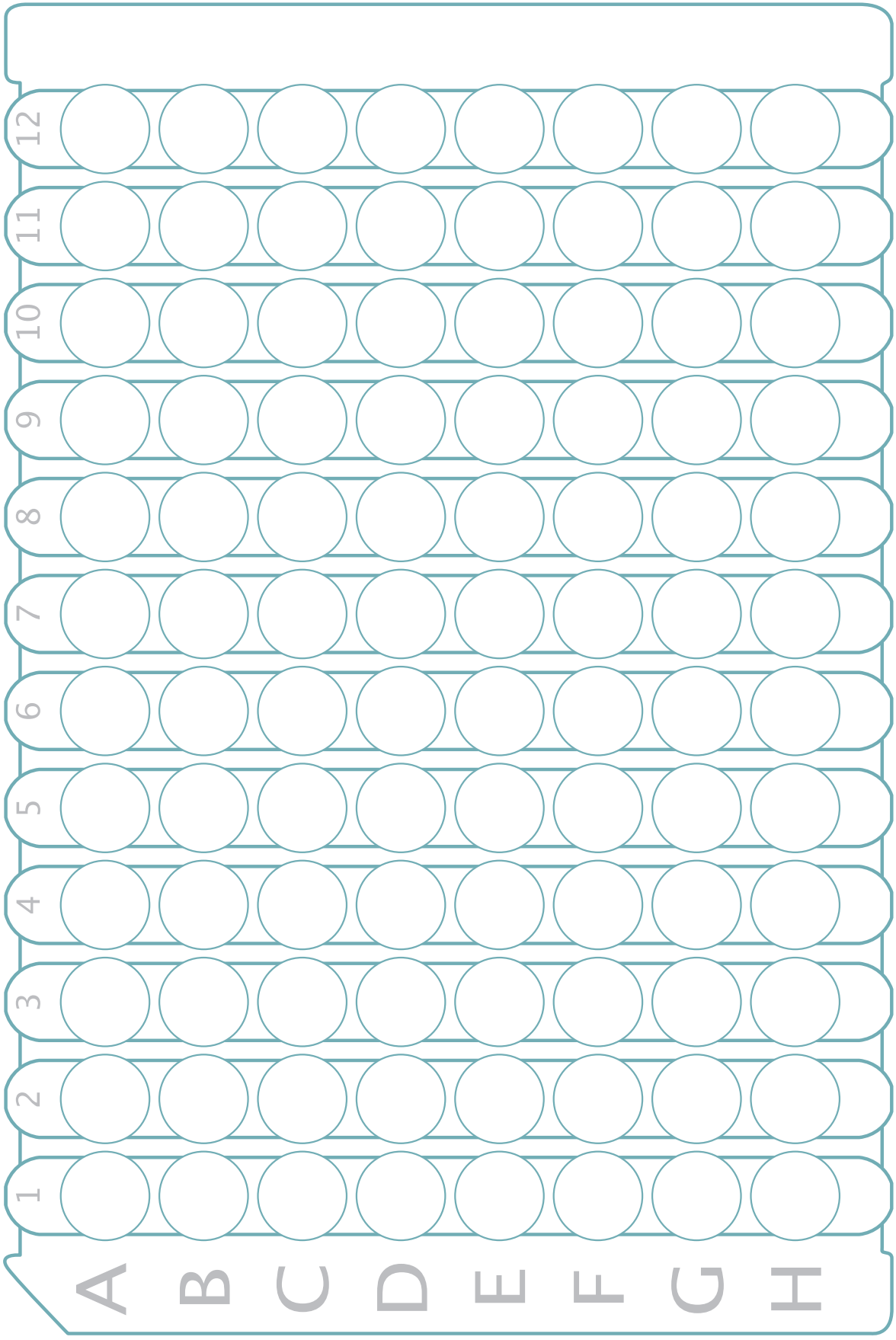
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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