Quantikine® ELISA

Human Survivin Immunoassay

Catalog Number DSV00

For the quantitative determination of human Survivin concentrations in cell culture supernates, serum, plasma, 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

Survivin, also known as baculoviral IAP repeat-containing protein 5 (BIRC5) and apoptosis inhibitor 4 (API4), is a bi-functional protein implicated in the regulation of cell division and the suppression of apoptosis (1). It is proposed to have roles in tumor formation and tumor cell resistance to anti-cancer agents, and may act as a marker and prognostic indicator for certain cancers (2). It is a member of the inhibitor of apoptosis protein (IAP) gene family (3-5). The IAP family of proteins shares a common sequence motif, known as a baculovirus IAP repeat (BIR) domain (5). Survivin is approximately 34 kDa and contains a single BIR domain and an extended carboxy-terminal α-helical coil that forms a bowtie-shaped homodimer (6, 7).

Survivin expression is regulated in a cell cycle-dependent manner with maximum expression occurring during the G2/M phase of the cell cycle (8). It is a component of the chromosomal passenger complex (CPC). The CPC has been implicated in the regulation of several mitosis-related events including chromosome alignment, histone modification, and cytokinesis (9). Other non-enzymatic members of the CPC include inner centromere protein (INCEP) and Dasra B/Borealin (10, 11). The CPC regulates the targeting and activity of the Aurora B serine/threonine kinase, an enzyme critical for the proper execution of cytokinesis (12).

In addition to its role as a mitotic regulator, Survivin also functions as an inhibitor of apoptosis by suppressing cell death initiated by both the intrinsic and extrinsic apoptosis pathways (3, 7, 13). The molecular basis of Survivin's anti-apoptotic mechanism continues to be intensely investigated. In addition to direct interaction with caspases (14), Survivin has been reported to interact with a number of other proteins. These include binding and inhibiting the pro-apoptotic mitochondrial protein SMAC/Diablo, and stabilizing XIAP by preventing ubiquitination and subsequent XIAP proteasomal degradation (15, 16).

Survivin is dramatically over-expressed in many cancers and the expression in these tumors can correlate with an increased rate of tumor recurrence and resistance to therapy (17-19). The presence of Survivin in urine may also act as a marker for bladder cancer (20, 21).

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

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human Survivin has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any Survivin present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked polyclonal antibody specific for human Survivin 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 Survivin 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, 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 Survivin Microplate	893225	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human Survivin.	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 Survivin Standard	893227	2 vials of recombinant human Survivin in a buffered protein solution with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human Survivin Conjugate	893226	21 mL of a polyclonal antibody specific for human Survivin conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-9	895167	11 mL of a buffered protein solution with preservatives. *May contain a precipitate. Warm to room temperature, and mix gently to dissolve. If the precipitate does not completely dissolve, mix well during use.*	
Calibrator Diluent RD5-33	895813	21 mL of a concentrated buffered protein solution with preservatives. *For cell culture supernate/urine samples. Use diluted 1:6 in this assay.*	
Calibrator Diluent RD6-47	895570	21 mL of a buffered protein solution 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.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 250 mL and 500 mL graduated cylinders.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- **Polypropylene** test tubes for dilution of standards.
- Human Survivin Controls (optional; R&D Systems®, Catalog # QC23).

PRECAUTIONS

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.*
Icteric samples are not recommended for use in this assay.

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.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

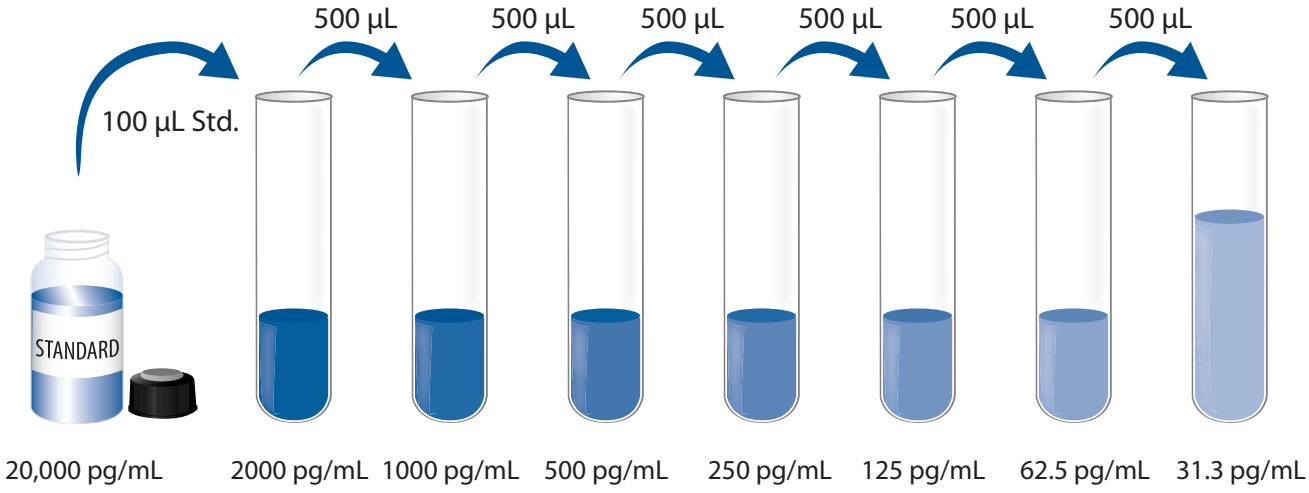
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to 480 mL of deionized or distilled water to prepare 500 mL of Wash Buffer.

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 RD5-33 (diluted 1:6) - *For cell culture supernate and urine samples.* Add 20 mL of Calibrator Diluent RD5-33 to 100 mL of deionized or distilled water to prepare 120 mL of Calibrator Diluent RD5-33 (diluted 1:6).

Human Survivin Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human Survivin Standard with deionized or distilled water. This reconstitution produces a stock solution of 20,000 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.

Use polypropylene tubes. Pipette 900 µL of Calibrator Diluent RD5-33 (diluted 1:6) (*for cell culture supernate/urine samples*) or Calibrator Diluent RD6-47 (*for serum/plasma samples*) into the 2000 pg/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 2000 pg/mL standard 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, controls, and samples be assayed in duplicate.

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 RD1-9 to each well. *Warm the Assay Diluent to room temperature, and mix well if precipitate is present.*
4. Add 100 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 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 Survivin 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.

CALCULATION OF RESULTS

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

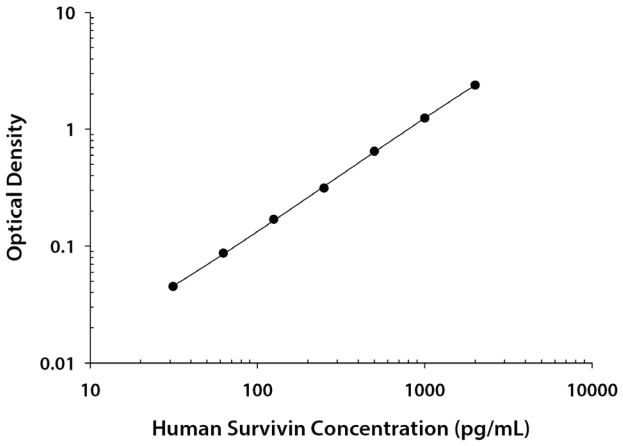
Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Survivin 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

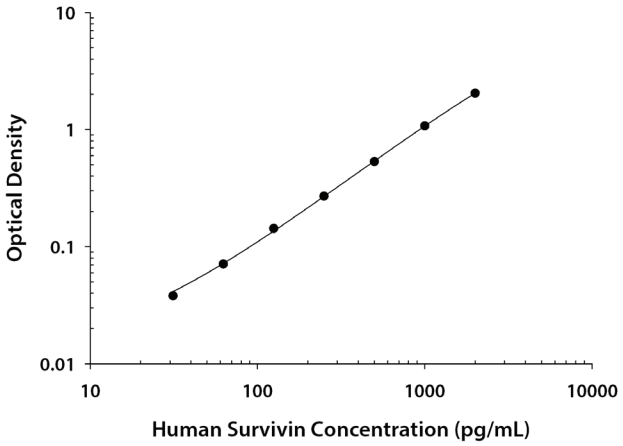
These standard curves are provided for demonstration only. A standard curve should be generated for each set of samples assayed.

CELL CULTURE SUPERNATE/URINE ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.018 0.018	0.018	—
31.3	0.062 0.063	0.063	0.045
62.5	0.102 0.107	0.105	0.087
125	0.185 0.189	0.187	0.169
250	0.330 0.331	0.331	0.313
500	0.661 0.668	0.665	0.647
1000	1.257 1.263	1.260	1.242
2000	2.392 2.408	2.400	2.382

SERUM/PLASMA ASSAY



(pg/mL)	O.D.	Average	Corrected
0	0.018 0.019	0.019	—
31.3	0.057 0.057	0.057	0.038
62.5	0.088 0.091	0.090	0.071
125	0.160 0.163	0.162	0.143
250	0.284 0.294	0.289	0.270
500	0.546 0.558	0.552	0.533
1000	1.066 1.120	1.093	1.074
2000	2.030 2.098	2.064	2.045

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/URINE ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	137	398	841	164	445	879
Standard deviation	6.41	27.3	44.2	14.6	39.4	63.8
CV (%)	4.7	6.9	5.3	8.9	8.9	7.3

SERUM/PLASMA ASSAY

	Intra-Assay Precision			Inter-Assay Precision		
Sample	**1**	**2**	**3**	**1**	**2**	**3**
n	20	20	20	40	40	40
Mean (pg/mL)	190	513	982	197	529	1060
Standard deviation	7.78	17.4	53.8	18.8	37.3	59.9
CV (%)	4.1	3.4	5.5	9.5	7.1	5.7

RECOVERY

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

Sample Type	Average % Recovery	Range
Cell culture media (n=4)	97	88-109%
Serum (n=4)	93	85-109%
EDTA plasma (n=4)	96	89-109%
Heparin plasma (n=4)	96	87-110%

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of human Survivin were serially diluted with 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	106	101	100	97
	Range (%)	103-113	95-110	95-105	93-105
1:4	Average % of Expected	111	100	98	100
	Range (%)	107-115	91-111	93-108	90-105
1:8	Average % of Expected	109	99	92	100
	Range (%)	98-116	91-108	89-96	89-108
1:16	Average % of Expected	106	94	89	91
	Range (%)	100-110	88-111	86-94	81-100

SENSITIVITY

Eighty-seven assays were evaluated and the minimum detectable dose (MDD) of human Survivin ranged from 1.58-9.96 pg/mL. The mean MDD was 4.44 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 Survivin produced at R&D Systems®.

SAMPLE VALUES

Serum/Plasma/Urine - Thirty-six serum, thirty-six of each plasma type, and eleven urine samples from apparently healthy volunteers were evaluated for the presence of human Survivin in this assay. No medical histories were available for the donors used in this study. No detectable levels were observed.

Cell Culture Supernates:
Human peripheral blood cells (1 x 10^6 cells/mL) were cultured in DMEM supplemented with 5% 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 for 1 and 5 days. Aliquots of the cell culture supernates were removed and assayed for levels of human Survivin.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Unstimulated	ND	ND
Stimulated	ND	82.1

ND=Non-detectable

BLIN-1 human Pre-B leukemia cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum. An aliquot of the cell culture supernate was removed, assayed for human Survivin, and measured 291 pg/mL.

MDA-MB-231 human breast cancer cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum until confluent. An aliquot of the cell culture supernate was removed, assayed for human Survivin, and measured 196 pg/mL.

MCF-7 human breast cancer cells were cultured in F12 and DMEM supplemented with 10% fetal bovine serum and 2 mM L-glutamine until confluent. An aliquot of the cell culture supernate was removed, assayed for human Survivin, and measured 148 pg/mL.

N1186 human T cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 10 ng/mL recombinant human IL-2 for 5 days. An aliquot of the cell culture supernate was removed, assayed for human Survivin, and measured 81.9 pg/mL.

SPECIFICITY

This assay recognizes natural and recombinant human Survivin.

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

Recombinant human:

Caspase-3
Caspase-7
Caspase-9
cIAP-1
Granzyme B
Livin α
MEK-1
P300
SMAC
TRAF-2
XIAP

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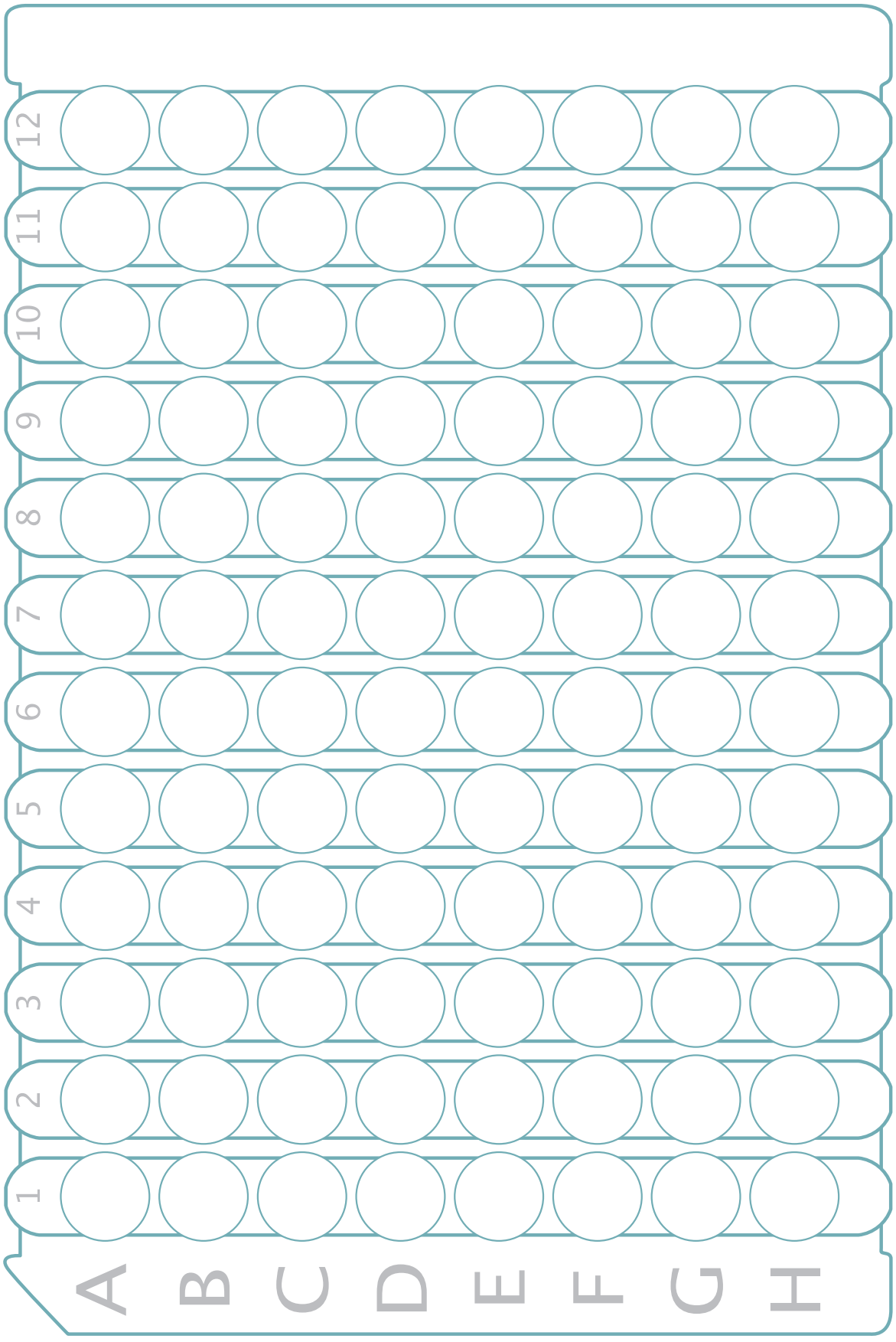
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



NOTES