Parameter™

Prostaglandin E_2 Assay

Catalog Number KGE004B
SKGE004B
PKGE004B

For the quantitative determination of Prostaglandin E_2 (PGE_2) 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

Prostaglandins (PG), thromboxanes, and leukotrienes belong to the class of prostanoid fatty acid derivatives of arachidonic acid. Arachidonic acid is liberated from membrane phospholipids by the action of phospholipases, metabolized into PGG_2 and PGH_2 by the cyclooxygenases COX-1 and COX-2, and converted into PGE_2 by prostaglandin E synthetase (PGES) (1-3). PGE_2 is found in many bodily fluids and is inactivated in the lungs and liver by prostaglandin dehydrogenase and cytochrome P-450 monooxygenases. COX and PGES exist in different isoforms that are constitutively active or inducible by inflammatory stimuli. PGE_2 synthesis can be blocked by corticosteroids that inhibit the phospholipases or by nonsteroidal anti-inflammatory drugs (NSAIDs) that inhibit the cyclooxygenases (4, 5). PGE_2 is produced by a wide variety of tissues, including bronchiolar, gastrointestinal, vascular, uterine, and bladder smooth muscle, fetal ductus arteriosus, placenta, brain, renal macula densa cells, testicular Leydig cells, mesenchymal stem cells, monocytes, and macrophages (6-17). Increased amounts of PGE_2 are produced in several pathologic conditions, including inflammation and arthritis, fever, tissue injury, endometriosis, and a wide variety of cancers (1, 7, 18-21).

The broad range of tissues that produce PGE_2 and multiple differentially expressed receptors and isoforms predict the complexity of PGE_2-induced effects. In the kidney, this includes stimulation of renin release and both promotion and inhibition of salt and water absorption (10, 15, 16). PGE_2 promotes either smooth muscle relaxation or contraction depending on the tissue (10, 22, 23). It is important in normal joint physiology and is a principal mediator of the inflammatory response to tissue damage (18, 19, 24). It sensitizes peripheral nociceptive nerves and exaggerates inflammatory and neuropathic pain by direct inhibition of spinal cord glycinergic neurotransmission (25). PGE_2 also plays a role in hippocampal synaptic plasticity and febrile responses (1, 8). PGE_2 activity is linked to the synthesis and release of several hormones and the fetal hypothalamus-pituitary-adrenal axis (7, 26). PGE_2 also stimulates tumor cell proliferation and differentiation as well as tumor-associated neovascularization (27, 28).

The Parameter™ Prostaglandin E_2 Immunoassay is a 3.5 hour forward sequential competitive enzyme immunoassay designed to measure PGE_2 in cell culture supernates, serum, plasma, and urine.

PRINCIPLE OF THE ASSAY

This assay is based on the forward sequential competitive binding technique in which PGE_2 present in a sample competes with horseradish peroxidase (HRP)-labeled PGE_2 for a limited number of binding sites on a mouse monoclonal antibody. PGE_2 in the sample is allowed to bind to the antibody in the first incubation. During the second incubation, HRP-labeled PGE_2 binds to the remaining antibody sites. Following a wash to remove unbound materials, a substrate solution is added to the wells to determine the bound enzyme activity. The color development is stopped, and the absorbance is read at 450 nm. The intensity of the color is inversely proportional to the concentration of PGE_2 in the sample.

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, incubation time or temperature, and kit age can cause assay variability.
- 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 this assay, 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.
- Pre-rinse the pipette tips when pipetting.
- Pipette standards and samples to the bottom of the wells. Add all other reagents to the side of the wells to avoid contamination.
- 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. Gently tap the side of the plate to ensure thorough mixing.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at ≤ -20 °C in a manual defrost freezer.
Do not use past kit expiration date.

PART	PART #	CATALOG # KGE004B	CATALOG # SKGE004B	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Goat Anti-mouse Microplate	892575	1 plate	6 plates	96 well polystyrene microplate (12 strips of 8 wells) coated with a goat anti-mouse polyclonal antibody.	Return unused wells to the storage bag. May be stored at 2-8 °C for up to 1 month.*
PGE_2 Standard	893377	1 vial	6 vials	PGE_2 in a buffer with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Aliquot and store at ≤ -20 °C for up to 1 month in a manual defrost freezer.*
PGE_2 Conjugate	893375	1 vial	6 vials	6 mL/vial of PGE_2 conjugated to horseradish peroxidase with red dye and preservatives.	May be stored for up to 1 month at 2-8 °C.*
Primary Antibody Solution	893376	1 vial	6 vials	6 mL/vial of a mouse monoclonal antibody to PGE_2 in a buffer with blue dye and preservatives.	
Calibrator Diluent RD5-56	895612	2 vials	12 vials	21 mL/vial of a buffered protein base with preservatives.	
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	12.5 mL/vial of stabilized hydrogen peroxide.	
Color Reagent B	895001	1 vial	6 vials	12.5 mL/vial of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895926	1 vial	6 vials	11 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.

KGE004B contains sufficient materials to run an ELISA on one 96 well plate.
SKGE004B (SixPak) contains sufficient materials to run ELISAs on six 96 well plates.

This kit is also available in a PharmPak (R&D Systems®, Catalog # PKGE004B). 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 or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Indomethacin (Sigma, Catalog # I7378 or equivalent).
- PGE_2 Controls (optional; R&D Systems®, Catalog # QC142).

PRECAUTIONS

This assay is temperature sensitive. Room temperature is defined as 18-23 °C.

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.

Care should be taken when handling the PGE_2 Standard because of the known and unknown effects of prostaglandins.

SAMPLE PREPARATION

All samples require a 3-fold dilution. A suggested 3-fold dilution is 150 µL of sample + 300 µL of Calibrator Diluent RD5-56.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Samples containing mouse or rat IgG may interfere with this assay.

Cell Culture Supernates - Remove particulates by centrifugation. 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 heparin or EDTA 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.*
Do not use lipemic samples.

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

***To inhibit prostaglandin synthesis by COX-2, indomethacin should be added to serum and plasma collection tubes immediately following draw (to a final concentration of approximately 10 µg/mL).**

REAGENT PREPARATION

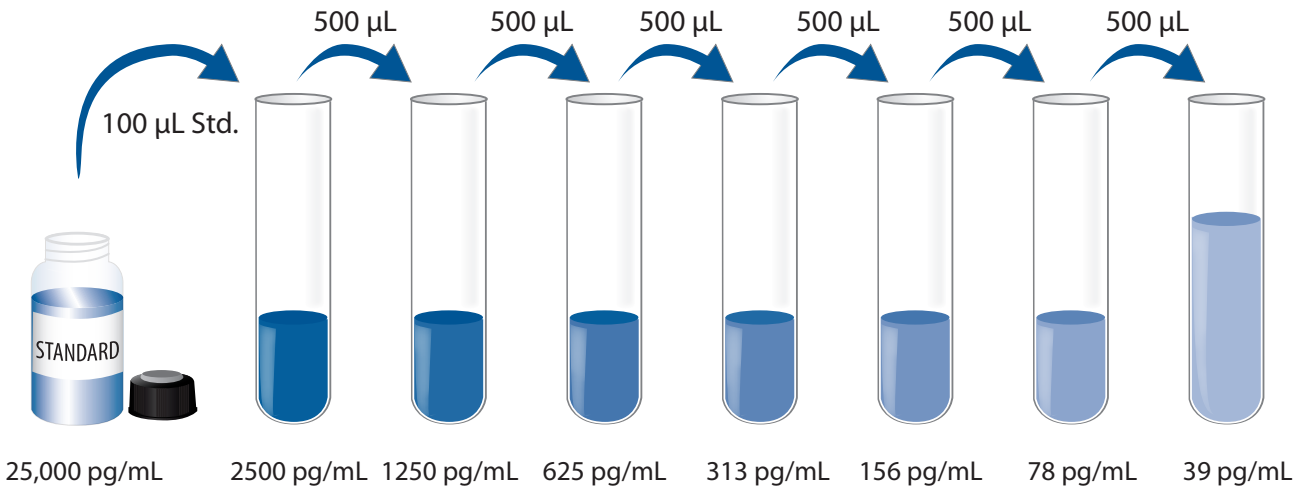
Bring all reagents to room temperature before use.

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.

PGE_2 Standard - Refer to the vial label for reconstitution volume. Reconstitute the PGE_2 Standard with deionized or distilled water. This reconstitution produces a stock solution of 25,000 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 900 µL of Calibrator Diluent RD5-56 into the 2500 pg/mL tube. Pipette 500 µL into the remaining tubes. Use the 25,000 pg/mL standard stock to produce a dilution series (below). Mix each tube thoroughly and change pipette tips between each transfer. The 2500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-56 serves as the zero standard (B0) (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature (18-23 °C) before use. It is recommended that all samples, controls, and standards 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 200 µL of Calibrator Diluent RD5-56 to the non-specific binding (NSB) wells.
4. Add 150 µL of Calibrator Diluent RD5-56 to the zero standard (B_0) wells.
5. Add 150 µL of standard, control, or sample* to the remaining wells.
6. Add 50 µL of the Primary Antibody Solution to each well **(excluding the NSB wells)**. All wells except the NSB wells will now be blue in color.
7. Securely cover with a plate sealer, and incubate for 1 hour 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.
8. **Do not wash the plate.** Add 50 µL of PGE_2 Conjugate to each well. All wells except the NSB wells will now be violet in color.
9. Cover with a new plate sealer, and incubate for 2 hours at room temperature on the shaker.
10. 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.
11. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
12. Add 100 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.
13. 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 or 570 nm. If wavelength correction is not available, subtract readings at 540 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 require dilution. See Sample Preparation section.

CALCULATION OF RESULTS

Average the duplicate readings for each standard, control, and sample then subtract the average NSB 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 a linear y-axis against the concentration on a logarithmic x-axis and draw the best fit curve through the points on the graph. Do not include the B_0 in the standard curve.

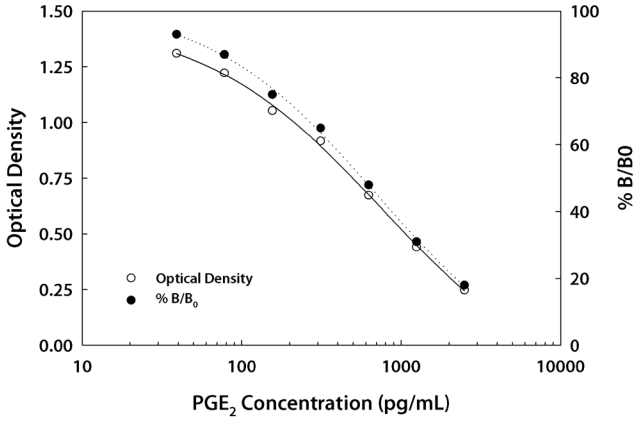
If desired, % B/B_0 can be calculated by dividing the corrected OD for each standard or sample by the corrected B_0 O.D. and multiplying by 100.

Calculate the concentration of PGE_2 corresponding to the mean absorbance from the standard curve.

Since 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	% B/B_0
NSB	0.009 0.009	0.009	—	—
0 (B_0)	1.404 1.419	1.412	1.403	—
39	1.304 1.333	1.319	1.310	93
78	1.212 1.250	1.231	1.222	87
156	1.051 1.073	1.062	1.053	75
313	0.910 0.939	0.925	0.916	65
625	0.657 0.707	0.682	0.673	48
1250	0.448 0.452	0.450	0.441	31
2500	0.249 0.265	0.257	0.248	18

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)	234	895	1456	253	835	1331
Standard deviation	21	45	89	33	82	120
CV (%)	9.0	5.0	6.1	12.9	9.9	9.0

RECOVERY

The recovery of PGE_2 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	84-106%
Serum* (n=4)	97	86-114%
EDTA plasma* (n=4)	93	82-108%
Heparin plasma* (n=4)	94	81-111%
Urine* (n=4)	99	81-111%

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

SENSITIVITY

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of PGE_2 ranged from 16.0-41.4 pg/mL. The mean MDD was 30.9 pg/mL.

The MDD was determined by subtracting two standard deviations from the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

LINEARITY

To assess the linearity of the assay, samples spiked with high concentrations of PGE_2 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)	Urine* (n=4)
1:2	Average % of Expected	100	99	104	108	93
	Range (%)	92-107	94-105	94-115	93-115	89-101
1:4	Average % of Expected	100	101	99	104	90
	Range (%)	94-107	95-107	97-106	96-113	85-96
1:8	Average % of Expected	101	104	103	102	92
	Range (%)	89-108	89-113	91-113	91-108	82-101
1:16	Average % of Expected	106	102	95	103	101
	Range (%)	101-113	87-127	80-116	93-115	92-107

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

SAMPLE VALUES

Serum/Plasma/Urine - Samples from apparently healthy volunteers were evaluated for the presence of PGE_2 in this assay. No medical histories were available for the donors used in this study.

Sample Type	Mean of Detectable (pg/mL)	% Detectable	Range (pg/mL)
Serum (n=31)	389	90	ND-2116
EDTA plasma (n=31)	414	94	ND-2052
Heparin plasma (n=31)	406	87	ND-2297
Urine (n=30)	796	67	ND-1850

ND=Non-detectable

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. Aliquots of the cell culture supernate were removed and assayed for levels of PGE_2.

Condition	Day 1 (pg/mL)	Day 5 (pg/mL)
Stimulated	4666	10,680
Unstimulated	ND	ND

ND=Non-detectable

SPECIFICITY

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 PGE_2 control were assayed for interference. No interference was observed. Cross-reactivity is listed below.

Compound	Cross-reactivity
PGE_3	14.5%
$PGF_{2\alpha}$	7.6%
$PGF_{1\alpha}$	7.4%
PGE_1	5.3%
6-keto-$PGF_{1\alpha}$	4.2%
PGA_2	0.55%
PGB_1	< 0.1%
TXB_2	< 0.1%
Arachidonic acid	< 0.1%
Arachidonylethanolamide	< 0.1%

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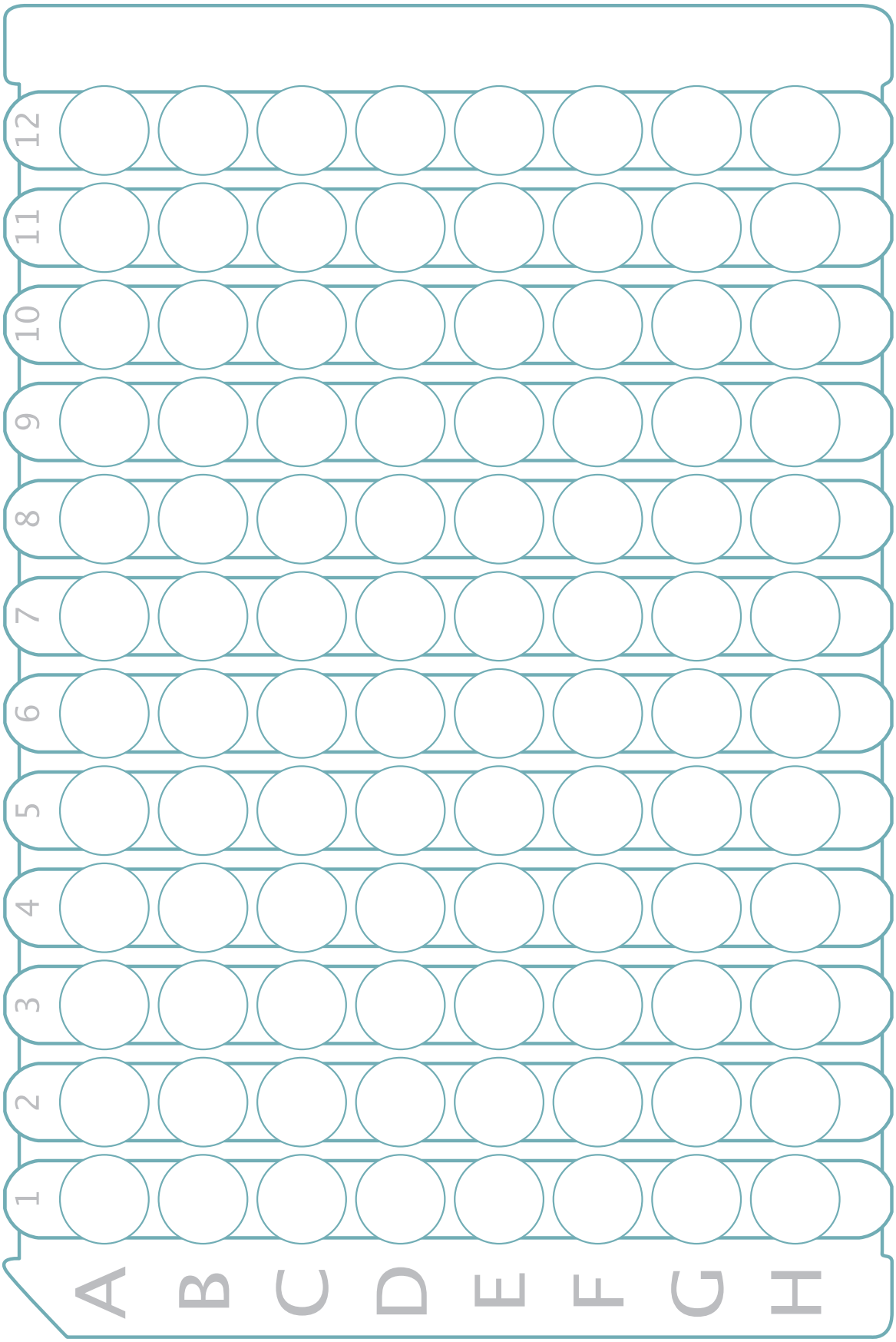
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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