# 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.

# TABLE OF CONTENTS

## SECTION PAGE




**Manufactured and Distributed by:**

**USA** R&D Systems, Inc.
614 McKinley Place NE, Minneapolis, MN 55413
**TEL:** 800 343 7475 612 379 2956
**FAX:** 612 656 4400
**E-MAIL:** info@bio-techne.com

**Distributed by:**

**Europe | Middle East | Africa** Bio-Techne Ltd.
19 Barton Lane, Abingdon Science Park
Abingdon OX14 3NB, UK
**TEL:** +44 (0)1235 529449
**FAX:** +44 (0)1235 533420
**E-MAIL:** info.emea@bio-techne.com

**China** Bio-Techne China Co., Ltd.
Unit 1901, Tower 3, Raffles City Changning Office,
1193 Changning Road, Shanghai PRC 200051
**TEL:** +86 (21) 52380373 (400) 821-3475
**FAX:** +86 (21) 52371001
**E-MAIL:** info.cn@bio-techne.com

# 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 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** | 895209 | 2 vials | 12 vials | 11 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** | 895032 | 2 vials | 12 vials | 6 mL/vial of 2N 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). Refer to the PharmPak Contents section for specific vial counts.

## PHARMPAK CONTENTS

Each PharmPak contains reagents sufficient for the assay of 50 microplates (96 wells/plate). The package inserts supplied are the same as those supplied in the single kit packs and because of this, a few minor differences related to the number of reagents and their container sizes should be noted.

- Sufficient material is supplied to perform at least 50 standard curves; reuse of each vial may be required. The number of vials, and the number of standard curves obtained per vial will vary with the analyte.
- Wash Buffer 25X Concentrate is bulk packed in 125 mL bottles containing 100 mL.
  **Note:** *Additional wash buffer is available for purchase (R&D Systems®, Catalog # WA126).*

The reagents provided in this PharmPak are detailed below.

| PART | PART # | QUANTITY |
|---|---|---|
| Goat Anti-mouse Microplate | 892575 | 50 plates |
| $PGE_2$ Standard* | 893377 | 25 vials |
| $PGE_2$ Conjugate | 893375 | 50 vials |
| Primary Antibody Solution | 893376 | 50 vials |
| Calibrator Diluent RD5-56 | 895612 | 100 vials |
| Wash Buffer Concentrate | 895126 | 9 bottles |
| Color Reagent A | 895000 | 50 vials |
| Color Reagent B | 895001 | 50 vials |
| Stop Solution | 895032 | 100 vials |
| Plate Sealers | N/A | 100 sheets |

**If additional standard vials are needed, contact Technical Service at techsupport@bio-techne.com*

## 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 (Tocris™, Catalog # 1708)
- $PGE_2$ 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 SDS 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

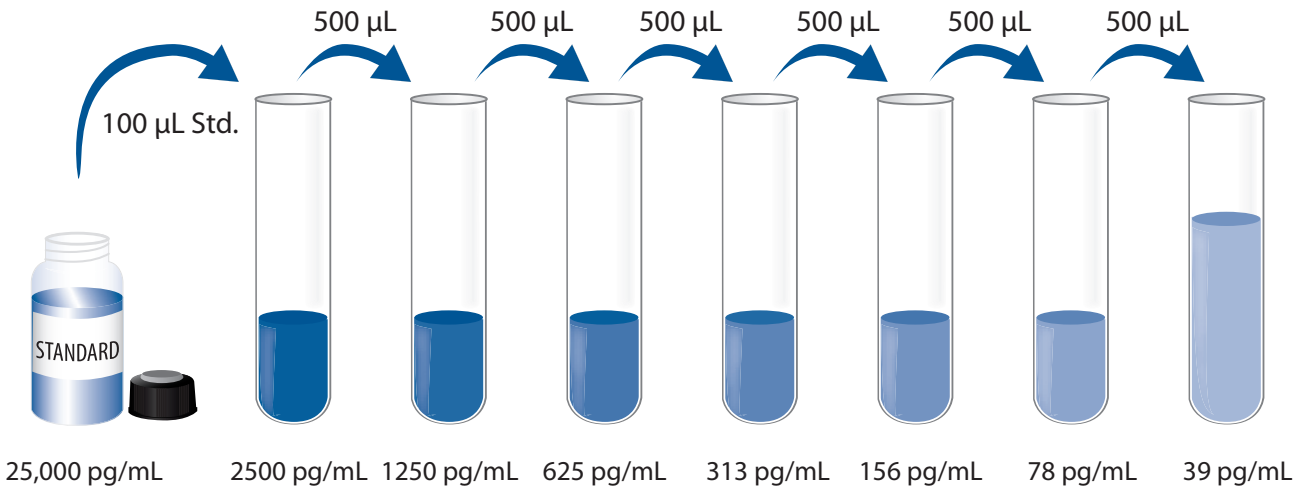
**Bring all reagents to room temperature before use.**

**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 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.

**$PGE_2$ 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 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 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.

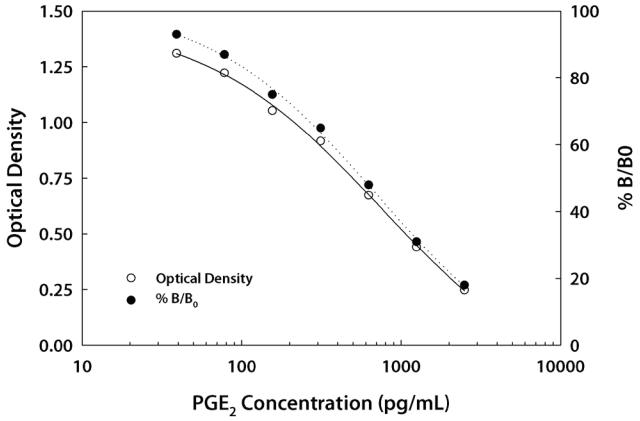
If desired, % $B/B_0$ can be calculated by dividing the corrected OD for each standard or sample by the corrected $B_0$ O.D. and multiplying by 100.

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<br>0.009 | 0.009 | — | — |
| 0 ($B_0$) | 1.404<br>1.419 | 1.412 | 1.403 | — |
| 39 | 1.304<br>1.333 | 1.319 | 1.310 | 93 |
| 78 | 1.212<br>1.250 | 1.231 | 1.222 | 87 |
| 156 | 1.051<br>1.073 | 1.062 | 1.053 | 75 |
| 313 | 0.910<br>0.939 | 0.925 | 0.916 | 65 |
| 625 | 0.657<br>0.707 | 0.682 | 0.673 | 48 |
| 1250 | 0.448<br>0.452 | 0.450 | 0.441 | 31 |
| 2500 | 0.249<br>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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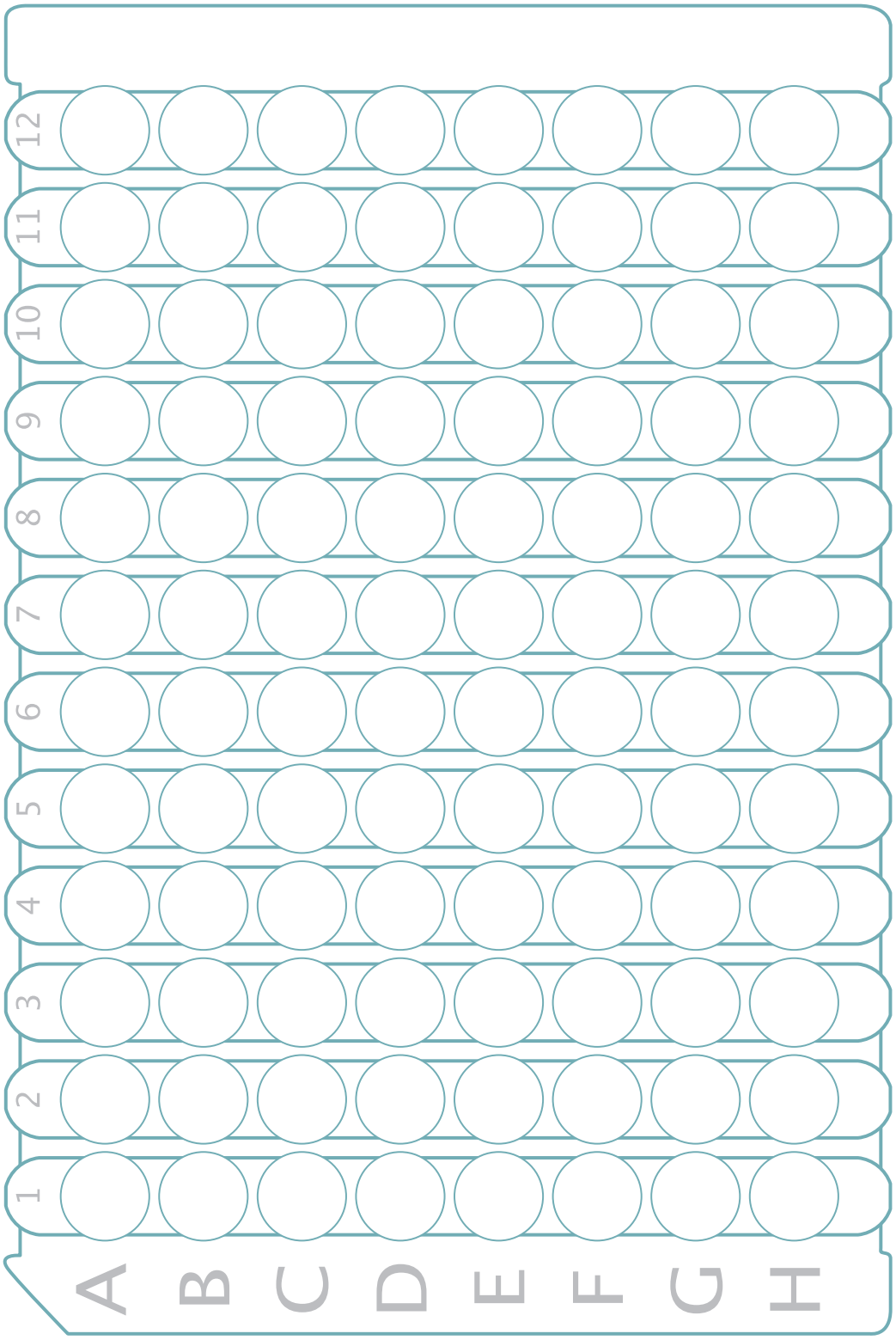
## PLATE LAYOUT

Use this plate layout to record standards and samples assayed.

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