StressMarq Biosciences INC.

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StressXpress®

Prostaglandin E2 EIA (Multi-format) Kit

Catalog# SKT-207 (96-Well Kit)

Quantitative colorimetric measurement of PGE2

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GENERAL INFORMATION

Materials Supplied

Catalog Number	Reagent	Quantity	Description
SKC-207A	Coated White 96 Well Plates	1 Each	Clear plastic microtiter plate coated with goat anti-mouse IgG.
SKC-207B	Prostaglandin E2 Standard	70 µL	Prostaglandin E2 at 20,000 pg/mL in a special stabilizing solution. **Must be stored at -20°C**
SKC-207C	StressXpress® Prostaglandin E2 Antibody	3 mL	A mouse monoclonal antibody specific for Prostaglandin E2.
SKC-207D	StressXpress® Prostaglandin E2 Conjugate	3 mL	A Prostaglandin E2-peroxidase conjugate in a special stabilizing solution. **Must be stored at -20°C.**
SKC-207E	Assay Buffer	28 mL	Ready to use Assay Buffer..
SKC-207F	Wash Buffer Concentrate	30 mL	A 20X concentrate that should be diluted with deionized water or distilled water.
SKC-207G	TMB Solution	11 mL	-
SKC-207H	Stop Solution	5 mL	A 1M solution of hydrochloric acid. CAUSTIC.
SKC-207I	Plate Sealer	1 Each	-

If any of the items listed above are damaged or missing, please contact our Customer Service department at (250) 294-9065. We cannot accept any returns without prior authorization.

! **WARNING: Not for human or animal disease diagnosis or therapeutic drug use.**

Precautions

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete booklet should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers Wash Buffers, containing sodium azide will inhibit color production from the enzyme. Make sure all buffers used for samples are azide free. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 9.

Storage

The unopened kit should be stored at -20°C.
Once opened the kit can be stored at 4°C up to the expiration date on the kit label, except for the PGE_2 Standard and PGE_2 Conjugate. These must be stored at -20°C. The frozen PGE_2 Conjugate can be freeze-thawed multiple times.

Materials Needed But Not Supplied

- Distilled or deionized water.
- Repeater pipet with disposable tips capable of dispensing 25 µL and 100µL.

- A microplate shaker.
- Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.
- Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

Please read this booklet completely prior to using the product.

FOR RESEARCH USE ONLY.

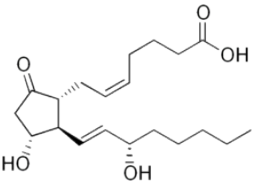
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTRODUCTION

Background

Eicosanoid signal transduction pathways are highly conserved and are involved in a number of physiological processes. Prostaglandins are synthesized from arachidonic acid by cyclooxygenase (COX)-1 or -2, which convert the acid into PGH2. This is further processed by cytosolic or microsomal prostaglandin synthases to become PGE_2 or one of several other prostanoids (1-3). Prostacyclin is the major cyclooxygenase product in blood vessel walls and it is present in inflammatory fluids in similar concentrations to PGE_2. Prostacyclin is a potent vasodilator and is more potent than PGE_2 in producing hyperalgesia (4). PGE_2 is produced by a wide variety of tissues (5-14) and in several pathological conditions, including inflammation, arthritis, fever, tissue injury, endometriosis, and a variety of cancers (5,6).

Other biological actions of PGE_2 include vasodilation, modulation of sleep/wake cycles, and facilitation of human immunodeficiency virus replication. It elevates cAMP levels, stimulates bone resorption, and has thermoregulatory effects. It has been shown to be a regulator of sodium excretion and renal hemodynamics (7-12).



Prostaglandin E_2

Assay Overview

The Prostaglandin E_2 (PGE_2) StressXpress EIA kit is designed to quantitatively measure PGE_2 present in serum, plasma, urine, saliva, cells, tissue, and tissue culture media samples. This EIA kit allows for the widest variations in sample size, sensitivity and assay timing of any PGE_2 kit. The protocol variations are outlined below. See Page 18-19 for data comparisons.

The Regular Format, which uses 50 µL of sample or standard, is shown on pages 10-11, gives results in 2.5 hours.

The Low Sample Volume Format, which uses 25 µL of sample or standard, is outlined on page 12-13, also gives results in 2.5 hours, but uses lower sample volumes.

The High Sensitivity Format, which uses 100 µL of sample or standard, is shown on page 14-15, also gives results in 2.5 hours, but is the highest sensitivity kit of any type available.

The Overnight Format, which uses 50 µL of sample or standard, is shown on page 16-17, and uses a primary overnight incubation at 4°C to fit your work flow. This format has similar sensitivity to the Regular Format outlined above.

PRE-ASSAY PREPARATION

Sample Types

Sample Types Validated:
Saliva, Urine, Serum, EDTA and Heparin Plasma and Tissue Culture Media

Prostaglandin E_2 (PGE_2) is identical across all species and we expect this kit may measure PGE_2 from sources other than human. The end user should evaluate recoveries of PGE_2 in other samples being tested. This assay has been validated for saliva, urine, serum, EDTA and heparin plasma samples and for tissue culture samples. A general cyclooxygenase inhibitor, such as meclofenamic acid or indomethacin at 15 µM should be added immediately after collection of any biological samples, such as serum and plasma. All samples should be frozen rapidly in dry ice/ethanol and stored at -80°C.
Samples containing visible particulates should be centrifuged prior to use. Severely hemolyzed samples should not be used in this kit. All samples with high lipid content may interfere with the measurement of PGE_2 and may be extracted as described below. An online resource for the extraction of bioactive lipids can be found at: http://lipidlibrary.aocs.org/topics/spe_alm/index.htm#ext.

Sample Values

The normal reference range for serum Prostaglandin E_2 (containing COX inhibitors) is 25-1,000 pg/mL. Typical normal mouse PGE_2 serum levels are 45-150 ng/mL. Normal 24-hour urine PGE_2 levels are between 400-620 ng/24 hours.

Sample Preparation

Serum and Plasma Samples

Serum and plasma samples should be diluted ≥ 1:10 with the supplied Assay Buffer prior running in the assay. Mouse serum and plasma samples need to be diluted ≥ 1:20 with the supplied Assay Buffer prior running in the assay to minimize any interference of mouse IgG on the assay.

Urine and Saliva Samples

Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay. Saliva samples should be diluted ≥ 1:2 with the supplied Assay Buffer prior running in the assay. See our Saliva Sample Handling Instructions available on this product's website page.

Tissue Culture Media

For measuring PGE_2 in tissue culture media (TCM), samples should be read off a standard curve generated in TCM. Samples may need to be diluted further in TCM. We have validated the assay using RPMI-1640.

Extracted Samples

See our Extraction Protocol Instructions available on this product's website page. The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Use all samples within 2 hours of preparation.

Reagent Preparation

Allow the kit reagents to thaw and come to room temperature for 30-60 minutes. We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine prostaglandin E_2 concentrations. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

Assay Buffer: Do not dilute the Assay Buffer.

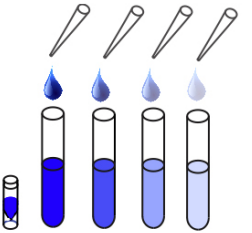
Wash Buffer :Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at room temperature for 3 months.

REGULAR FORMAT: Preparation and Protocol

Standard Preparation

Label test tubes as #1 through #8. Pipet 390 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #8. The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 10 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 200 µL of the Prostaglandin E_2 solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of Prostaglandin E_2 in tubes 1 through 8 will be 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 pg/mL.

Use all Standards within 2 hours of preparation.



	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	390	200	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Volume of Addition (µL)	10	200	200	200	200	200	200	200
Final Conc. (pg/mL)	500	250	125	62.5	31.25	15.625	7.813	3.906

Assay Protocol

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 50 µL of samples or standards into wells in the plate.
3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 µL of the StressXpress® Prostaglandin E_2 Conjugate to each well using a repeater pipet.
6. Add 25 µL of the StressXpress® Prostaglandin E_2 Antibody to each well, except the NSB wells, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 concentration for each sample.

LOW SAMPLE VOLUME FORMAT: Preparation and Protocol

Standard Preparation

Label test tubes as #1 through #7. Pipet 380 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #7. The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 20 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 200 µL of the Prostaglandin E_2 solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #7. The concentration of Prostaglandin E_2 in tubes 1 through 7 will be 1,000, 500, 250, 125, 62.5, 31.25, and 15.625 pg/mL.

Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer (µL)	380	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	10	200	200	200	200	200	200
Final Conc. (pg/mL)	1000	500	250	125	62.5	31.25	15.625

Assay Protocol

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 25 µL of samples or standards into wells in the plate.
3. Pipet 50 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 25 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 µL of the *StressXpress*® Prostaglandin E_2 Conjugate to each well using a repeater pipet.
6. Add 25 µL of the *StressXpress*® Prostaglandin E_2 Antibody to each well, except the NSB wells, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 concentration for each sample.

HIGH-SENSITIVITY FORMAT: Preparation and Protocol

Standard Preparation

Label test tubes as #1 through #9. Pipet 585 µL of Assay Buffer into tube #1 and 300 µL into tubes #2 to #9. The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 15 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 300 µL of the Prostaglandin E_2 solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #9. The concentration of Prostaglandin E_2 in tubes 1 through 9 will be 500, 250, 125, 62.5, 31.25, 15.625, 7.813, 3.906, and 1.953 pg/mL.

Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8	Std 9
Assay Buffer (µL)	585	300	300	300	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Volume of Addition (µL)	15	300	300	300	300	300	300	300	300
Final Conc. (pg/mL)	500	250	125	62.5	31.25	15.63	7.813	3.906	1.953

Assay Protocol

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 100 µL of samples or standards into wells in the plate.
3. Pipet 125 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 100 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 µL of the *StressXpress®* Prostaglandin E_2 Conjugate to each well using a repeater pipet.
6. Add 25 µL of the *StressXpress®* Prostaglandin E_2 Antibody to each well, except the NSB wells, using a repeater pipet.
7. Gently tap the sides of the plate to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and shake at room temperature for 2 hours. If the plate is not shaken signals bound will be approximately 40% lower.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 concentration for each sample.

OVERNIGHT FORMAT: Preparation and Protocol

Standard Preparation

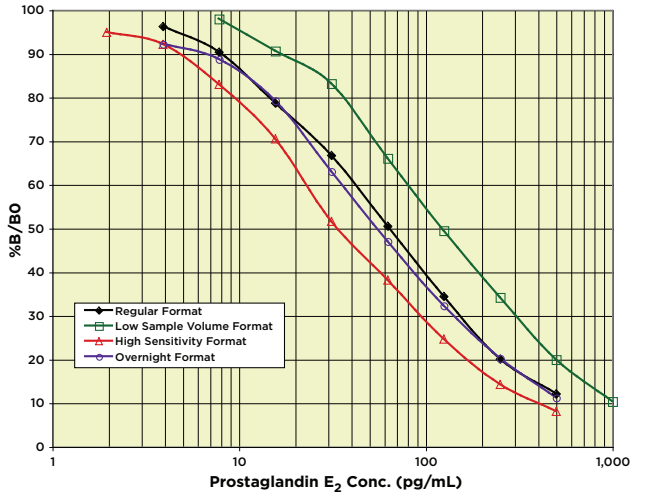
Label test tubes as #1 through #8. Pipet 390 µL of Assay Buffer into tube #1 and 200 µL into tubes #2 to #7. The Prostaglandin E_2 stock solution contains an organic solvent. Prerinse the pipet tip several times to ensure accurate delivery. Carefully add 10 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely. Take 200 µL of the Prostaglandin E_2 solution in tube #1 and add it to tube #2 and vortex completely. Repeat the serial dilutions for tubes #3 through #8. The concentration of Prostaglandin E_2 in tubes 1 through 8 will be 500, 250, 125, 62.5, 31.25, 15.625, 7.813 and 3.906 pg/mL.

Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7	Std 8
Assay Buffer (µL)	390	200	200	200	200	200	200	200
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Volume of Addition (µL)	10	200	200	200	200	200	200	200
Final Conc. (pg/mL)	500	250	125	62.5	31.25	15.625	7.813	3.906

Assay Protocol

1. Use the plate layout sheet on the back page to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to the foil pouch with desiccant. Seal the ziploc plate bag and store at 4˚C.
2. Pipet 50 µL of samples or standards into wells in the plate.
3. Pipet 75 µL of Assay Buffer into the non-specific binding (NSB) wells.
4. Pipet 50 µL of Assay Buffer into wells to act as maximum binding wells (B0 or 0 pg/mL).
5. Add 25 µL of the *StressXpress*® Prostaglandin E_2 Conjugate to each well using a repeater pipet.
6. Add 25 µL of the *StressXpress*® Prostaglandin E_2 Antibody to each well, except the NSB wells, using a repeater pipet.
7. Shake the plate in a plate shaker at room temperature for 15 minutes to ensure adequate mixing of the reagents. Cover the plate with the plate sealer and store at 4°C for 16-18 hours.
8. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
9. Add 100 µL of TMB Substrate to each well, using a repeater pipet.
10. Incubate the plate at room temperature for 30 minutes without shaking.
11. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
12. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
13. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E_2 concentration for each sample.


%B/B0
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80
70
60
50
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30
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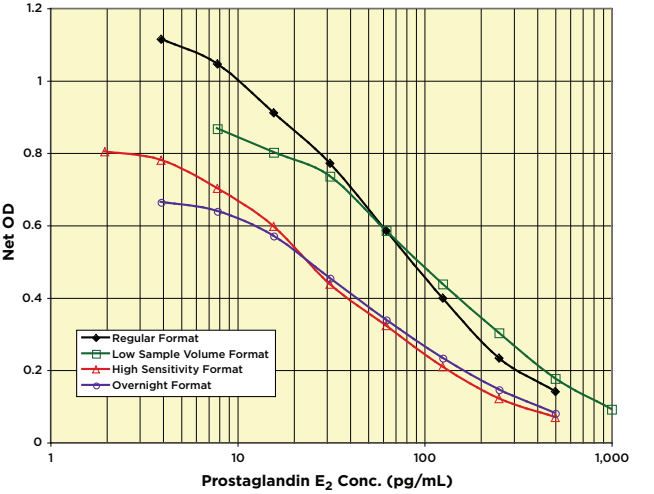
Prostaglandin E2 Conc. (pg/mL)
Regular Format
Low Sample Volume Format
High Sensitivity Format
Overnight Format


Net OD
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0.8
0.6
0.4
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1
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100
1,000
Prostaglandin E2 Conc. (pg/mL)
Regular Format
Low Sample Volume Format
High Sensitivity Format
Overnight Format

ANALYSIS

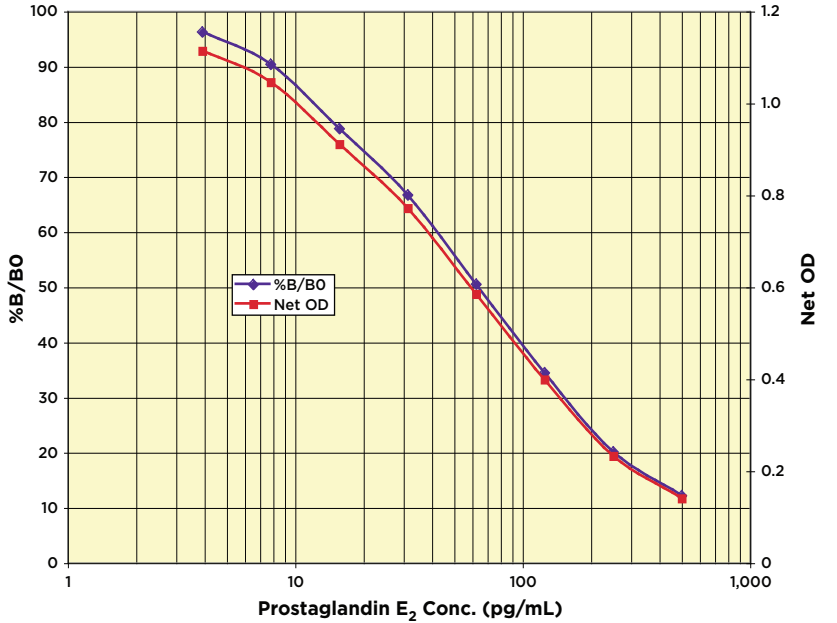
Calculation of Results

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using the 4PLC fitting routine on the plate reader, after subtracting the mean OD's for the NSB. The sample concentrations obtained, calculated from the %B/B0 curve, should be multiplied by the dilution factor to obtain neat sample values.

Typical Data

Sample	Mean OD	Net OD	% B/B0	PGE_2 Conc. (pg/mL)
NSB	0.061	0	-	-
Std 1	0.202	0.141	12.19	500
Std 2	0.294	0.233	20.14	250
Std 3	0.460	0.399	34.49	125
Std 4	0.646	0.585	50.56	62.5
Std 5	0.833	0.772	66.72	31.25
Std 6	0.972	0.911	78.74	15.625
Std7	1.107	1.046	90.41	7.813
Std 8	1.175	1.114	96.28	3.906
B0	1.218	1.157	100	0
Sample 1	0.464	0.403	34.83	121.8
Sample 2	1.030	0.969	83.75	12.28

Typical Regular Format Standard Curve



Always run your own standard curve for calculation of results.
Do not use this data.

Validation Data

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the B0 and standard #8. The detection limit was determined at two (2) standard deviations from the B0 along the standard curve.
Sensitivity was determined as 3.07 pg/mL.

The Limit of Detection for the assay was determined in a similar manner by

comparing the OD's for twenty runs for each of the zero standard and a low concentration human sample.
Limit of Detection was determined as 3.25 pg/mL.
We expect the High Sensitivity Format to give enhanced Sensitivity and LoD.

Linearity

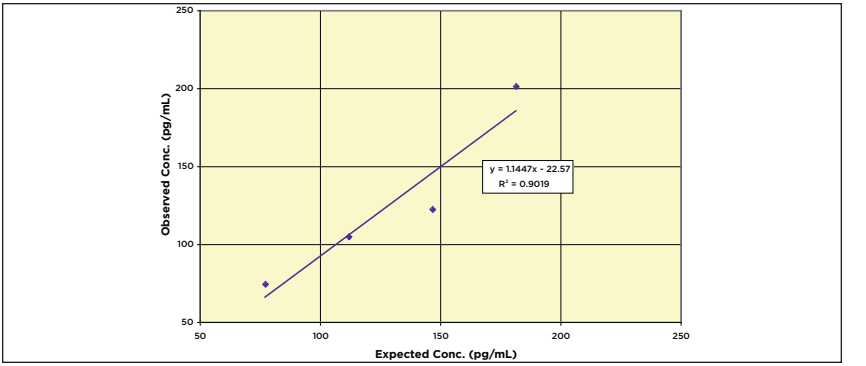
Linearity was determined in human plasma and urine samples by taking two diluted samples with known PGE_2 concentrations. A plasma sample with a high PGE_2 concentration of 216.4 pg/mL was mixed with one with a lower value of 42.5 pg/mL. A urine sample with a high PGE_2 concentration of 32.6 pg/mL was mixed with one with a lower value of 8.6 pg/mL. They were mixed in the ratios given below. The measured concentrations were compared to the expected values based on the ratios used.

Plasma Linearity

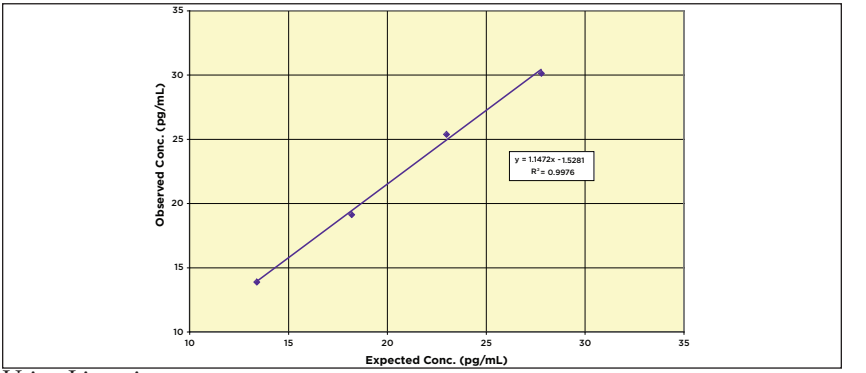
High Sample	Low Sample	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	201.1	181.6	110.8%
60%	40%	122.3	146.8	83.3%
40%	60%	104.7	112.0	93.5%
20%	80%	74.3	77.3	96.1%
			Mean Recovery	95.9%

Urine Linearity

High Sample	Low Sample	Observed Conc. (pg/mL)	Expected Conc. (pg/mL)	% Recovery
80%	20%	30.1	27.8	108.3
60%	40%	25.4	23.0	110.2
40%	60%	19.1	18.2	105.0
20%	80%	13.9	13.4	103.3
			Mean Recovery	106.7%



Plasma Linearity



Urine Linearity

Intra Assay Precision

Three human samples were diluted with Assay Buffer and run in replicates of 20 in an assay. The mean and precision of the calculated Prostaglandin E_2 concentrations were:

Sample	Prostaglandin E_2 Concentration (pg/mL)	%CV
1	11.7	12.3
2	98.6	6.3
3	131.2	4.9

Inter Assay Precision

Three human samples were diluted with Assay Buffer and run in duplicates in seventeen assays run over multiple days by four operators. The mean and precision of the calculated Prostaglandin E_2 concentrations were:

Sample	Prostaglandin E_2 Concentration (pg/mL)	%CV
1	12.3	8.8
2	100.5	8.1
3	134.7	9.8

Sample Values

Eight human serum samples that did not contain COX inhibitors were tested in the assay. Neat sample were diluted 1:20-1:50 in Assay Buffer and adjusted values ranged from 652 to 4,170 pg/mL with an average of 2,126 pg/mL. Ten human plasma samples that did not contain COX inhibitors were tested in the assay. Neat sample were diluted 1:20-1:50 in Assay Buffer and adjusted values ranged from 219 to 4,328 pg/mL with an average of 1,717 pg/mL. Eight normal human urine samples were diluted 1:10- 1:20 in Assay Buffer and adjusted values ranged from 56.9 to 326 pg/mL with an average of 149.9 pg/mL

Cross Reactivity

The following cross reactants were tested in the assay and calculated at the 50% binding point.

Eicosanoid	Cross Reactivity (%)
Prostaglandin E_2	100%
Prostaglandin E_1	27.28%
Prostaglandin F_{2a}	0.33%
Thromboxane B_2	< 0.02%
6-keto-Prostaglandin F_{1a}	< 0.02%
15-keto-Prostaglandin E_1	< 0.02%
16,16-dimethyl-Prostaglandin E_2	< 0.02%
Arachidonic Acid	< 0.02%

Interferents

A variety of solvents were tested as possible interfering substances in the assay. Organic solvents such as DMSO, Dimethylformamide (DMF), methanol and ethanol were tested in the assay at 0.1%. DMSO and DMF caused a 1.2% and 0.8% decrease in measured PGE_2 levels, whereas methanol and ethanol caused an increase of 2.5% and 4.6% in measured PGE_2 levels. A solvent only control should be run by the end user when appropriate.

Hemoglobin at 0.02 mg/dL caused a 1% decrease in measured PGE_2 levels.

Elevated lipids will also interfere with the measurement of PGE_2. Follow the extraction recomendations described on page 9.

References

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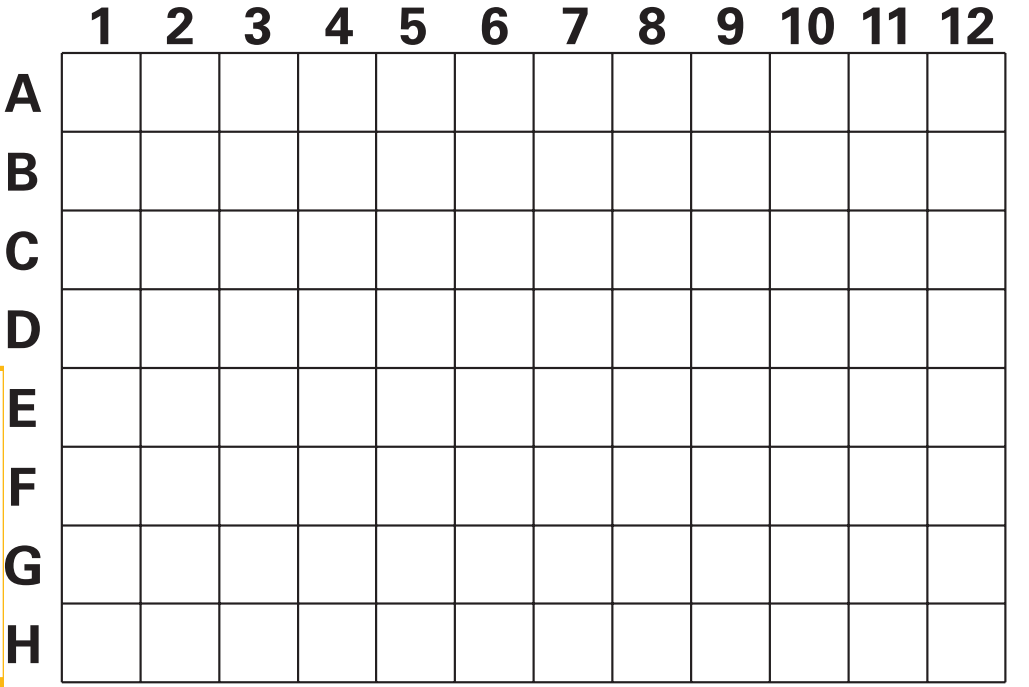
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