DRG® Prostaglandin E2 (EIA-5194)

Revised 9 Mar. 2011 rm (Vers. 2.1) **RUO**

Please use only the valid version of the package insert provided with the kit.

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

Species Independent

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

1. Moncada, S., Ferriera, SH. & Vane, JR. (1979). "Pain and inflammatory mediators." In Anti-Inflammatory Drugs. Handbook of Experimental Pharmacology, 50/II. Pp. 588-616. Vane, J.R. & Ferreira, S.H. Berlin, New York: Springer.
2. Vane, JR. (1971). "Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs." Nature, 231, 232-235.3.
3. Willis, AL. (1969). "Release of histamine, kinin and prostaglandins during carrageenin-induced inflammation in the rat." In Prostaglandins, Peptides and Amines. Pp. 31-38. Ed. Mantegazza, P. & Horton, E.W. London: Academic Press.
4. Higgs, GA., Cardinal, DC., Moncada, S. & Vane, JR. (1979). "Microcirculatory effects of prostacyclin (PGI_2) in the hamster cheek pouch." Microvascular Res., 18, 245-254.
5. Kargman, S. et al. "Mechanism of selective inhibition of human prostaglandin G/H synthase-1 and -2 in intact cells" (1996) Biochem Pharmacol. 52(7):1113-25
6. Thun MJ, Namboodiri MM, Heath CW Jr. "Aspirin use and reduced risk of fatal colon cancer." New Engl. J. Med. 1991; 325: 1593–6.
7. Richardson PD and Withrington PG, , "The vasodilator actions of isoprenaline, histamine, prostaglan din E_2, glucagon and secretin on the hepatic arterial vascular bed of the dog." Brit. J. Pharmacol., (1976) 57: 581-588.
8. O. Hayaishi, "Sleep-Wake Regulation by Prostaglandins D_2 and E_2." J. Biol. Chem., (1988) 263: 14593- 14596.
9. S. Kuno, et al., "Prostaglandin E_2, a seminal constituent, facilitates the replication of acquired immune deficiency syndrome virus in vitro. "Proc. Natl. Acad. Sci., USA, (1986) 83: 3487-3490.
10. D.L. Bareis, et al., "Bradykinin stimulates phospholipid methylation, calcium influx, prostaglandin formation, and cAMP accumulation in human fibroblasts". Proc. Natl. Acad. Sci., USA, (1983) 80: 2514-2518.
11. L.G. Raisz, et al., "Effect of prostaglandin endoperoxides and metabolites on bone resorption in vitro." Nature, (1977) 267: 532-534.
12. C.R. Long, Kinoshita Y, Knox FG., "Prostaglandin E_2 induced changes in renal blood flow, renal interstitial hydrostatic pressure and sodium excretion in the rat." Prostaglandins, (1990) 40: 591-601.

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1 ASSAY PRINCIPLE

The Prostaglandin E_2 (PGE_2) Immunoassay kit is designed to quantitatively measure PGE_2 present in serum, plasma, urine, saliva and tissue culture media samples.

Please read the complete kit insert before performing this assay.

A PGE_2 standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with an antibody to capture mouse IgG. A PGE_2-peroxidase conjugate is added to the standards and samples in the wells. The binding reaction is initiated by the addition of a monoclonal antibody to PGE_2 to each well. After a 2 hour incubation, the plate is washed and substrate is added. The substrate reacts with the bound PGE_2-peroxidase conjugate. After a short incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450nm wavelength. The concentration of the PGE_2 in the sample is calculated, after making suitable correction for the dilution of the sample, using software available with most plate readers

2 SUPPLIED COMPONENTS

Coated Clear 96 Well Plate
A clear plastic microtiter plate coated with goat anti-mouse IgG.
1 each

Prostaglandin E_2 Standard
Prostaglandin E_2 at 20,000 pg/mL in a special stabilizing solution.
70 µL

Prostaglandin E_2 Antibody
A mouse monoclonal antibody specific for Prostaglandin E_2.
3 mL

Prostaglandin E_2 Conjugate
A Prostaglandin E_2-peroxidase conjugate in a special stabilizing solution.
3 mL

Assay Buffer
One plate kit uses a ready-to-use Assay Buffer.
28 mL

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL

TMB Substrate
15 mL

Stop Solution
A 1M solution of hydrochloric acid. CAUSTIC.
5 mL

Plate Sealer
1 each

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3 STORAGE INSTRUCTIONS

All components of this kit should be stored at -20°C until the expiration date of the kit.

Once opened the kit can be stored at 4°C for up to 3 months.

4 OTHER MATERIALSREQUIRED

Distilled or deionized water.

A microplate shaker.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm, preferably with correction at between 570 and 590 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.

5 PRECAUTIONS

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

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

6 SAMPLE TYPES

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 particulate should be centrifuged prior to using.

Severely hemolyzed samples should not be used in this kit.

All samples containing lipids may interfere with the measurement of PGE_2. Samples containing high lipid content may be extracted as described below. A useful online resource for the extraction of bioactive lipids can be found at: http://lipidlibrary.aocs. org/topics/spe_alm/index.htm#ext .

Prostaglandin E_2 is identical across all species and we expect this kit may measure prostaglandin E_2 from sources other than human. The end user should evaluate recoveries of prostaglandin E_2 in other samples being tested.

7 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 Samples

Urine samples should be diluted ≥ 1:8 with the supplied Assay Buffer prior running in the assay.

Saliva Samples

Saliva samples should be diluted ≥ 1:2 with the supplied Assay Buffer prior running in the assay.

Tissue Culture Media

For measuring prostaglandin E_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

We have a detailed Extraction Protocol available upon request.

The ethanol concentration in the final Assay Buffer dilution added to the well should be <5%.

Use all samples within 2 hours of preparation.

8 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.

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.

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Standard Preparation

Label six test tubes as #1 through #6.
Pipet 475 µL of Assay Buffer into tube #1 and 250 µL into tubes #2 to #6.
The Prostaglandin E_2 stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.
Carefully add 25 µL of the Prostaglandin E_2 stock solution to tube #1 and vortex completely.
Take 250 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 #6.
The concentration of Prostaglandin E_2 in tubes 1 through 6 will be 1,000, 500, 250, 125, 62.5, and 31.25 pg/mL.

Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (µL)	**475**	250	250	250	250	250
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (µL)	**25**	250	250	250	250	250
Final Conc (pg/mL)	1,000	500	250	125	62.5	31.25

9 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 (Bo or 0 pg/mL).
5. Add 25 µL of the Prostaglandin E_2 Conjugate to each well using a repeater or multichannel pipet.
6. Add 25 µL of the Prostaglandin E_2 Antibody to each well, **except the NSB wells**, using a repeater or multichannel 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 4 times with the wash buffer. Tap the plate dry on absorbent towels.
9. Add 150 µL of the TMB Substrate to each well, using a repeater or a multichannel 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 or a multichannel 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.

10 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	0.101	0	-	-
Standard 1	0.293	0.192	9.3	1,000
Standard 2	0.516	0.415	20.2	500
Standard 3	0.809	0.708	34.5	250
Standard 4	1.198	1.097	53.4	125
Standard 5	1.554	1.453	70.7	62.5
Standard 6	1.786	1.685	82.0	31.25
B0	2.156	2.055	100.0	0
Sample 1	0.393	0.292	14.2	693.9
Sample 2	1.553	1.452	70.7	61.8

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

Conversion Factor: 100 pg/mL of prostaglandin E_2 is equivalent to 283.7 pM.

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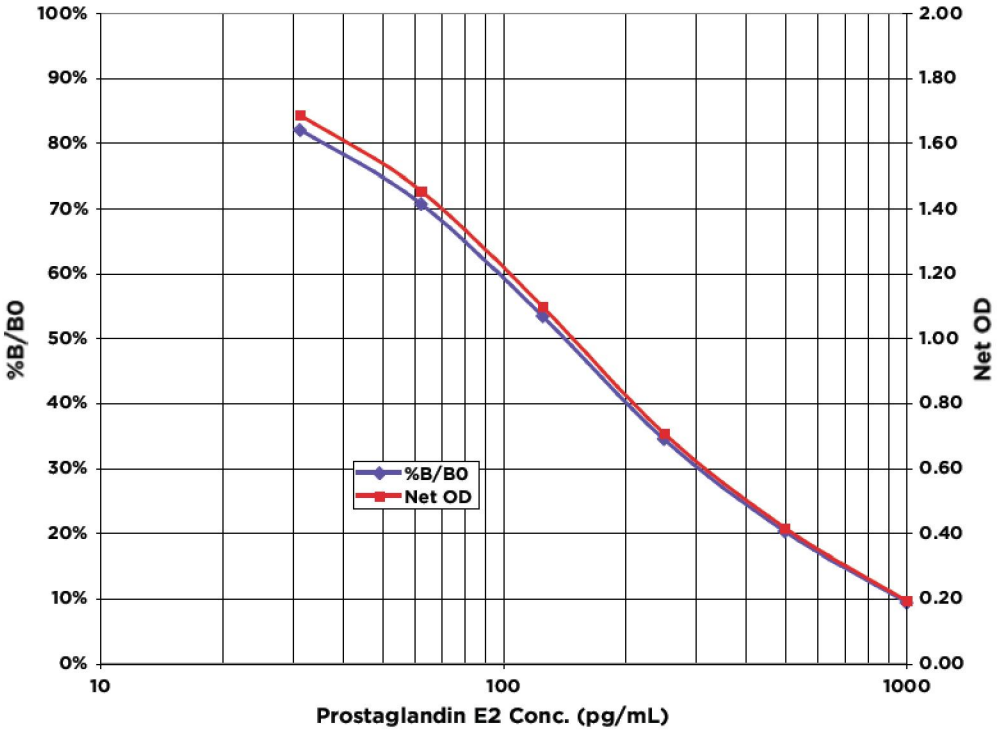
Typical Standard Curves



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

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11 LIMITED WARRANTY

DRG warrants that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 48 hours of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

Version 2011-02-28~rm