



DRG[®] Prostaglandin E2 HS (EIA-5203)



As of 15 Mar. 2011 rm (Vers. 1.1)



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., Ferreira, 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 (PGI₂) 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, prostaglandin E₂, 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 D₂ and E₂." J. Biol. Chem., (1988) 263: 14593- 14596.
9. S. Kuno, et al., "Prostaglandin E₂, 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 E₂ 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 E₂ (PGE₂) High Sensitivity Immunoassay kit is designed to quantitatively measure PGE₂ present in serum, plasma, urine, saliva and tissue culture media samples. Please read the complete kit insert before performing this assay. A PGE₂ 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 PGE₂-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 PGE₂ to each well. After an overnight incubation at 4°C, the plate is washed and substrate is added. The substrate reacts with the bound PGE₂-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 450 nm wavelength.

The concentration of the PGE₂ 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

Clear plastic microtiter plate coated with goat anti-mouse IgG.
1 each

Prostaglandin E₂ Standard

Prostaglandin E₂ at 20,000 pg/mL in a special stabilizing solution.
70 µL

Prostaglandin E₂ High Sensitivity Antibody

A mouse monoclonal antibody specific for Prostaglandin E₂.
3 mL

Prostaglandin E₂ Conjugate

A Prostaglandin E₂-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 1 M 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 MATERIALS REQUIRED

Distilled or deionized water.

A microplate shaker.

A 4 °C refrigerator

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 PGE₂. 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 E₂ is identical across all species and we expect this kit may measure prostaglandin E₂ from sources other than human. The end user should evaluate recoveries of prostaglandin E₂ in other samples being tested.

7 SAMPLE PREPARATION

Serum and Plasma Samples

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



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Urine Samples

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

Saliva Samples

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

Tissue Culture Media

For measuring prostaglandin E₂ 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 E₂ 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.

Standard Preparation

Label six test tubes as #1 through #6.

Pipet 588 μL of Assay Buffer into tube #1 and 300 μL into tubes #2 to #6.

The Prostaglandin E₂ stock solution contains an organic solvent. Pre-rinse the pipet tip several times to ensure accurate delivery.

Carefully add 12 μL of the Prostaglandin E₂ stock solution to tube #1 and vortex completely.

Take 300 μL of the Prostaglandin E₂ 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 E₂ in tubes 1 through 6 will be 400, 200, 100, 50, 25, and 12.5 pg/mL..

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Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Assay Buffer (μL)	588	300	300	300	300	300
Addition	Stock	Std 1	Std 2	Std 3	Std 4	Std 5
Vol of Addition (μL)	12	300	300	300	300	300
Final Conc (pg/mL)	400	200	100	50	25	12.5

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 E₂ Conjugate to each well using a repeater or multichannel pipet.
6. Add 25 μL of the Prostaglandin E₂ High Sensitivity Antibody to each well, **except the NSB wells**, using a repeater or multichannel pipet.
7. Cover the plate with the plate sealer and shake the plate for 15 minutes at room temperature.
8. Place the covered plate in a 4°C refrigerator for 16 hours.
9. The next morning take the plate from the refrigerator and wash the plate 4 times with the wash buffer. Tap the plate dry on absorbent towels.
10. Add 150 μL of the TMB Substrate to each well, using a repeater or a multichannel pipet.
11. Incubate the plate at room temperature for 30 minutes.
12. Add 50 μL of the Stop Solution to each well, using a repeater or a multichannel pipet.
13. Read the optical density generated from each well in a plate reader capable of reading at 450 nm.
14. Use the plate reader's built-in 4PLC software capabilities to calculate prostaglandin E₂ 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.

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Typical Data

Sample	Mean OD	Net OD	% B/B0	PGE ₂ Conc. (pg/mL)
NSB	0.069	0	-	-
Standard 1	0.397	0.328	12.8	400
Standard 2	0.713	0.644	25.1	200
Standard 3	1.220	1.151	44.9	100
Standard 4	1.683	1.614	63.0	50
Standard 5	2.196	2.127	83.1	25
Standard 6	2.319	2.250	87.1	12.5
B0	2.630	2.561	100.0	0
Sample 1	0.900	0.831	32.4	148.8
Sample 2	1.376	1.307	51.0	80.65

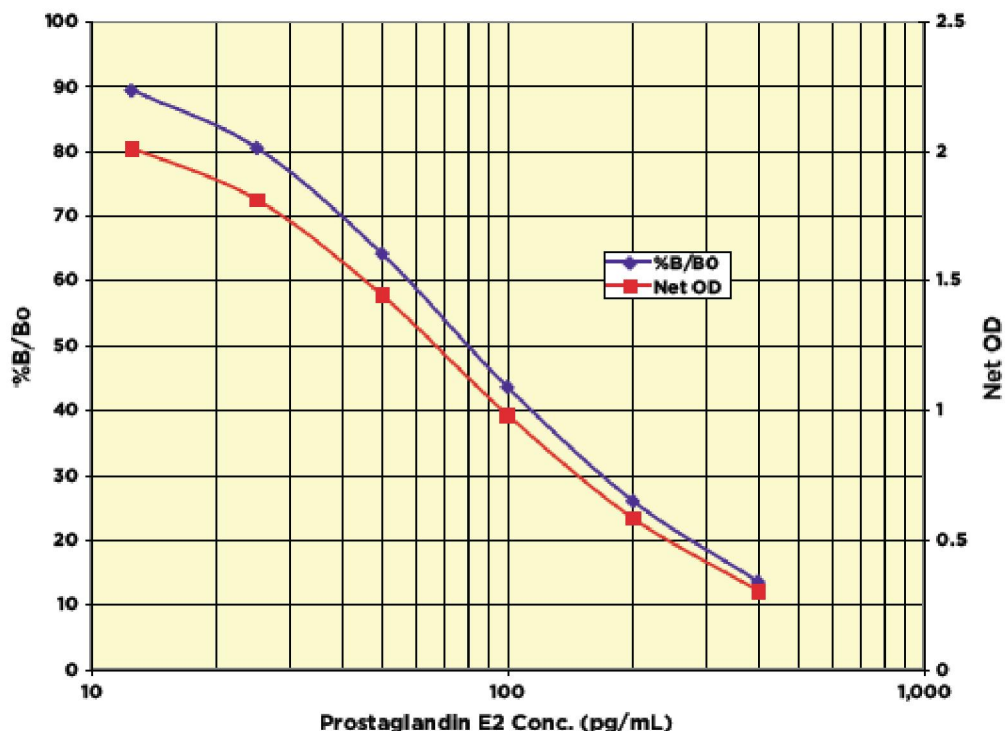
Always run your own standard curve for calculation of results. Do not use this data.

Conversion Factor: 100 pg/mL of prostaglandin E₂ 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.

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.

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