NEOGEN®
CORPORATION
ISO 9001:2008

Prostaglandin E_2 (Monoclonal) ELISA Kit Instructions

Please read all instructions carefully before beginning this assay

PRODUCT #406510
For research use only.

Storage Conditions:
Lyophilized conjugate: -20°C or less.
Do not freeze kit components.
Do not freeze reconstituted conjugate.

DESCRIPTION

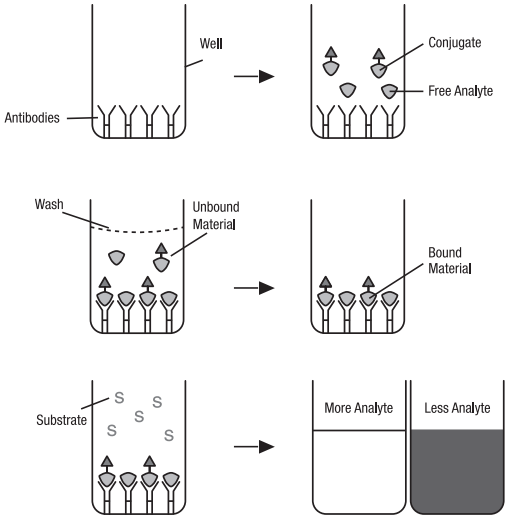
Prostaglandin E_2 (PGE_2) is derived from PGH_2 which in turn is synthesized from arachidonic acid through the cyclooxygenase pathway. Many cell types, such as epithelial cells, fibroblasts, and macrophages produce PGE_2. PGE_2 induces vasoactivity, modulates immune functions, regulates sleep-awake cycles and exhibits many other activities.

PRINCIPLE OF ASSAY

This is an ELISA (Enzyme-Linked ImmunoSorbent Assay) for the quantitative analysis of Prostaglandin E_2 levels in biological fluid. This test kit operates on the basis of competition between the enzyme conjugate and the PGE_2 in the sample for a limited number of binding sites on the antibody coated plate.

The sample or standard solution is first added to the microplate. Next, the diluted enzyme conjugate is added and the mixture is shaken and incubated at room temperature for one hour. During the incubation, competition for binding sites is taking place. The plate is then washed removing all the unbound material. The bound enzyme conjugate is detected by the addition of substrate which generates an optimal color after 30 minutes. Quantitative test results may be obtained by measuring and comparing the absorbance reading of the wells of the samples against the standards with a microplate reader at 650 nm. The extent of color development is inversely proportional to the amount of PGE_2 in the sample or standard. For example, the absence of PGE_2 in the sample will result in a bright blue color, whereas the presence of PGE_2 will result in decreased or no color development.

PRINCIPLE OF ASSAY (continued)



MATERIALS PROVIDED

1. ***EIA BUFFER:*** 30 mL. Provided to dilute enzyme conjugate and PGE_2 standards.
2. ***WASH BUFFER (10X):*** 20 mL. Dilute 10-fold with deionized water. Diluted wash buffer is used to wash all unbound enzyme conjugate, samples and standards from the wells after the one hour incubation.
3. ***K-BLUE SUBSTRATE:*** 20 mL. Stabilized 3,3', 5,5' Tetramethylbenzidine (TMB) plus Hydrogen Peroxide (H_2O_2) in a single bottle. It is used to develop the color in the wells after they have been washed. **LIGHT SENSITIVE**. Keep substrate refrigerated.
4. ***EXTRACTION BUFFER (5X):*** 30 mL. Dilute 5-fold with deionized water. This buffer is used for diluting extracted and non-extracted samples.
5. ***PROSTAGLANDIN E_2 ENZYME LYOPHILIZED CONJUGATE:*** Two vials of lyophilized PGE_2 horseradish peroxidase conjugate. Reconstitution with 75 µL of deionized water results in a 50:1 concentrate. Blue capped vials.
6. ***PROSTAGLANDIN E_2 STANDARD:*** 100 µL. PGE_2 standard provided at the concentration of 1 µg/mL. Green capped vial. Solution is viscous, take precaution when pipetting.
7. ***PROSTAGLANDIN E_2 ANTIBODY-COATED MICROPLATE:*** A 96 well Costar microplate precoated with monoclonal mouse antibody against PGE_2 The plate is ready for use as is. **DO NOT WASH!**

MATERIALS NEEDED BUT NOT PROVIDED

1. 300 mL deionized water to dilute wash buffer, extraction buffer and lyophilized conjugate.
2. Precision pipettes that range from 10 µL-1000 µL and disposable tips.

NOTE: If all or several strips are to be used at one time, it is suggested that a multichannel pipette be used.

3. Clean test tubes used to dilute the standards and conjugate.
4. Graduated cylinders to dilute and mix wash buffer and extraction buffer.
5. Microplate reader with 650 nm filter.
6. Plate cover or plastic film to cover plate during incubation.

OPTIONAL MATERIALS:

7. 1 N HCl or Neogen's Red Stop Solution.
8. Microplate shaker.

If performing an extraction on samples, the following will be required:

9. Methanol
10. Methyl formate
11. 0.1 M Sodium Phosphate buffer, pH 7.5
12. C_{18} Sep-Pak® column (Waters® Corporation)
13. Petroleum ether
14. Nitrogen gas
15. Vortex
16. Centrifuge

WARNINGS AND PRECAUTIONS

1. **DO NOT** use components beyond expiration date.
2. **DO NOT** mix any reagents or components of this kit with any reagents or components of any other kit. This kit is designed to work properly as provided.
3. **DO NOT** pipette reagents by mouth.
4. Always pour substrate out of the bottle into a clean test tube - **DO NOT** pipette out of the bottle. If pipette tip is unclean this could result in contamination of the substrate.
5. All specimens should be considered potentially infectious. Exercise proper handling precautions.
6. **DO NOT** smoke, eat or drink in areas where specimens or reagents are being handled.
7. Use aseptic technique when opening and removing reagents from vials and bottles.
8. Keep plate covered except when adding reagents, washing or reading.
9. Kit components should be refrigerated at all times when not in use. Lyophilized conjugate should be stored at -20°C until reconstituted.
10. Ensure that the conjugate is completely reconstituted before use. Each vial, when reconstituted, provides sufficient reagent to assay 64 wells (8 strips). If more than 64 wells are to be run, reconstitute both vials and pool the reconstituted conjugate.

PROCEDURAL NOTES

1. It is not necessary to allow reagents to warm to room temperature before use.
2. Desiccant bag must remain in foil pouch with unused strips. Keep zip-lock pouch sealed when not in use to maintain a dry environment.
3. Always use new pipette tips to pipette buffer, enzyme conjugate, standards and samples.
4. Before pipetting a reagent, rinse the pipette tip three times with that reagent (i.e. fill the tip with the desired amount of reagent and dispense back into the same vial - repeat 2 times). Now the tip is properly rinsed and ready to dispense the reagent into your well or test tube.
5. When pipetting into the wells, **DO NOT** allow the pipette tip to touch the inside of the well, or any of the reagents already in the well. This can result in cross contamination.
6. Standards and samples should be assayed in duplicate.
7. To quantitate, always run samples alongside a standard curve. If testing a sample that is not extracted, standards should be diluted in the same type of medium being tested. This medium should be known to be negative.
8. Gently mix specimens and reagents before use. Avoid vigorous agitation.
9. Before opening lyophilized conjugate vial, examine the vial to ensure that lyophilized materials have not been trapped in the cap. If material is trapped in the cap, gently tap the upright vial to dislodge the trapped material.
10. To reconstitute the lyophilized conjugate, add 75 µL of deionized water to vial. Rehydrate the conjugate by gently rotating the vial. Do not vortex or shake the contents. Avoid excess foaming. After the solid material has gone into solution, conjugate is ready for use. Write the date of reconstitution on the label. Concentrated, reconstituted conjugate has a shelf

life of at least 30 days when stored at 4°C.

11. The enzme conjugate is most stable in its concentrated form. Dilute only the volume necessary for the amount of strips currently being used.
12. Before taking an absorbance reading, wipe the outside bottom of the wells with a lint-free wiper to remove dust and fingerprints.

SAMPLE PREPARATION

This assay is non-species specific. Usually, urine and tissue culture supernatant can be assayed directly by diluting them with the diluted extraction buffer. Plasma and most other mediums will need to be extracted.

EXTRACTION OF PGE_2

1. Add 0.2 mL of methanol to 1 mL of biological fluid and vortex.
2. For tissue, homogenize it in 15% methanol in 0.1 M sodium phosphate buffer, pH 7.5 (100 mg in 1 mL methanol - buffer). Centrifuge the homogenate for five (5) minutes. Collect the supernatant in a clean tube.
3. Precondition the C_{18} Sep-Pak® column (Waters® Corporation) by washing the column with 2 mL of methanol followed by 2 mL of water.
4. Apply the above sample into the column and adjust the flow rate to 1 mL per minute. Reducing the flow rate to 0.5 mL per minute may increase extraction efficiencies. Some samples may clog the column. These samples may be diluted 1:3 or 1:6 in phosphate buffer (10 to 100 mM, pH~7.0) to improve the flow rate.
5. Wash the column with 2 mL of 15% methanol in water followed by 2 mL of petroleum ether.
6. The Prostaglandin is eluted with 2 mL of methyl formate.
7. Evaporate methyl formate eluate with a stream of nitrogen gas.
8. Dissolve the residue with 1 mL of diluted extraction buffer and assay 50 µL in duplicates.
9. The residue may be dissolved in less than 1 mL of diluted extraction buffer if sample concentration is suspected to be low. Values obtained must then be divided by the concentration factor in order to calculate final ng/mL concentration. If sample concentration is higher than the high range of the standard curve, the residue may be further diluted in step 8, and values obtained must then be multiplied by the dilution factor in order to calculate final ng/mL concentration.

NOTE: Extraction buffer must be diluted 5-fold with deionized water before use. Any precipitant present must be brought into solution before dilution.

TEST PROCEDURES

1. Standards should be prepared immediately before running the assay, as follows:

Standard	*Preparation*
A	stock solution 1 µg/mL (provided in green capped vial)
B	take 20 µL of A, add to 980 µL of EIA buffer and mix=20 ng/mL
C	take 200 µL of B, add to 1.8 mL of EIA buffer and mix=2 ng/mL
D	take 200 µL of C, add to 1.8 mL of EIA buffer and mix=0.2 ng/mL

Continue standard preparation following Scheme I.

SCHEME I

Standards	*ng/mL*	*EIA buffer (µL added)*	*B standard µL*	*C standard µL*	*D standard µL*
S_0	0	as is	-	-	-
S_1	0.1	500	-	-	500
S_2	0.2	-	-	-	as is
S_3	0.4	800	-	200	-
S_4	0.8	600	-	400	-
S_5	1	500	-	500	-
S_6	2	-	-	as is	-
S_7	4	800	200	-	-

2. Determine the number of wells to be used.
3. Dilute the reconstituted PGE_2 enzyme conjugate. Add 1 µL of enzyme conjugate into 50 µL total volume of EIA buffer for each well assayed. For the entire plate, add 110 µL of the Prostaglandin E_2 enzyme conjugate into 5.5 mL total volume of EIA Buffer. Mix the solution thoroughly.

NOTE: If more conjugate concentrate is needed than is contained in the first conjugate vial, reconstitute and use the second vial. Do not use the separate contents of both vials in the same assay as some assay variability may result. If the contents of both vials are required for an assay, pool the concentrated conjugates. Use the expiration date of the oldest reconstituted vial for the pool. Alternatively, prepare the necessary volumes of diluted conjugate and pool before using in the assay.

4. Add 50 µL of standards (S) or unknown (U) (some samples may require diluting) to the appropriate wells in duplicate.

See Scheme II for suggested template design.

5. Add 50 µL of the diluted enzyme conjugate to each well. Use 8-channel pipette or 12-channel pipette for rapid addition.
6. Mix by shaking the plate gently. A microplate shaker may be used.
7. Cover the plate with plastic film and incubate at room temperature for one hour.

NOTE: Keep plate away from drafts and temperature fluctuations.

8. Dilute concentrated wash buffer with deionized water (i.e. 20 mL of wash buffer plus 180 mL of deionized water). Mix thoroughly.
9. After incubation, dump out the contents of the plate. Tap out contents thoroughly on a clean lint-free towel.
10. Wash each well with 300 µL of the diluted wash buffer. Repeat for a total of four washings. An automated plate washer may be used, however, increase wash cycles from four to five.
11. Add 150 µL of substrate to each well. Use multichannel pipette for best results. Mix by shaking plate gently.
12. Incubate at room temperature for 30 minutes.
13. Gently shake plate before measuring optical density to ensure uniform color throughout each well.
14. Plate is read in a microplate reader at 650 nm. If a dual wavelength is used, set W_1 at 650 nm and W_2 at 490 nm.
15. If accounting for substrate background, use 2 to 8 wells as blanks with only substrate in the wells (150 µL/well). Subtract the average of these absorbance values from the absorbance values of the wells being assayed.

NOTE: Some microplate readers can be programmed to do these subtractions automatically when reading the plate. Consult your instrument manual.

OPTIONAL TEST PROCEDURES

16. Add 50-100 µL of 1 N HCl or Neogen's Red Stop Solution to each well to stop enzyme reaction.
17. Read plate at 450 nm, if 1N HCl solution was used. Read plate at 650 nm, if Neogen's Red Stop Solution was used.
18. Plot the standard curve and estimate the concentrations of the samples from the curve. See "CALCULATIONS."

NOTE: Absorbance readings will approximately double when stopped with acid. If absorbance readings are too high for measuring with your microplate reader, decrease the substrate incubation by approximately 10 minutes but no more than 15 minutes.

SCHEME II

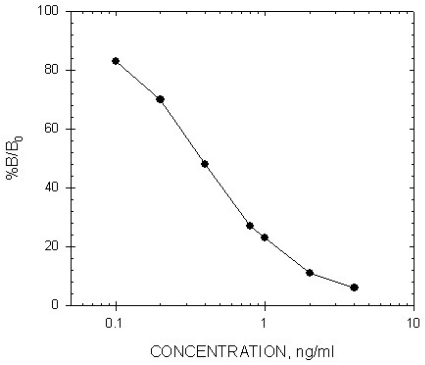
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*
A	$^{S}0$	$^{S}0$	$^{U}1$	$^{U}1$	$^{U}9$	$^{U}9$	$^{U}17$	$^{U}17$	$^{U}25$	$^{U}25$	$^{U}33$	$^{U}33$
B	$^{S}1$	$^{S}1$	$^{U}2$	$^{U}2$	$^{U}10$	$^{U}10$	$^{U}18$	$^{U}18$	$^{U}26$	$^{U}26$	$^{U}34$	$^{U}34$
C	$^{S}2$	$^{S}2$	$^{U}3$	$^{U}3$	$^{U}11$	$^{U}11$	$^{U}19$	$^{U}19$	$^{U}27$	$^{U}27$	$^{U}35$	$^{U}35$
D	$^{S}3$	$^{S}3$	$^{U}4$	$^{U}4$	$^{U}12$	$^{U}12$	$^{U}20$	$^{U}20$	$^{U}28$	$^{U}28$	$^{U}36$	$^{U}36$
E	$^{S}4$	$^{S}4$	$^{U}5$	$^{U}5$	$^{U}13$	$^{U}13$	$^{U}21$	$^{U}21$	$^{U}29$	$^{U}29$	$^{U}37$	$^{U}37$
F	$^{S}5$	$^{S}5$	$^{U}6$	$^{U}6$	$^{U}14$	$^{U}14$	$^{U}22$	$^{U}22$	$^{U}30$	$^{U}30$	$^{U}38$	$^{U}38$
G	$^{S}6$	$^{S}6$	$^{U}7$	$^{U}7$	$^{U}15$	$^{U}15$	$^{U}23$	$^{U}23$	$^{U}31$	$^{U}31$	$^{U}39$	$^{U}39$
H	$^{S}7$	$^{S}7$	$^{U}8$	$^{U}8$	$^{U}16$	$^{U}16$	$^{U}24$	$^{U}24$	$^{U}32$	$^{U}32$	$^{U}40$	$^{U}40$

CALCULATIONS

1. After the substrate background has been subtracted from all absorbance values, average all of your duplicate well absorbance values.
2. The average of your two S_0 values is now your B_0 value. (S_1 now becomes B_1, etc.)
3. Next, find the percent of maximal binding (%B/B_0 value). To do this, divide the averages of each standard absorbance value (now known as B_1 through B_7) by the B_0 absorbance value and multiply by 100 to achieve percentages.
4. Graph your standard curve by plotting the %B/B_0 for each standard concentration on the ordinate (y) axis against concentration on the abscissa (x) axis. Draw a curve by using a curve-fitting routine (i.e. 4-parameter or linear regression).
5. Divide the averages of each sample absorbance value by the B_0 value and multiply by 100 to achieve percentages.
6. Using the standard curve, the concentration of each sample can be determined by comparing the %B/B_0 of each sample to the corresponding concentration of PGE_2 standard.
7. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

TYPICAL STANDARD CURVE

PGE_2 in EIA Buffer



TYPICAL DATA

NOTE: ***"Typical data" is a representation. Variances in data will occur. Optical density readings may fluctuate during the shelf-life of the kit, but the %B/B_0 should remain comparable. Measuring wavelength: 650 nm***

Standard	*Standard Concentration (ng/mL)*	*Optical Density (Absorbance Value)*	*%B/B_0*
S_0 (B_0)	0	1.158	100
S_1 (B_1)	0.1	0.955	83
S_2 (B_2)	0.2	0.810	70
S_3 (B_3)	0.4	0.554	48
S_4 (B_4)	0.8	0.312	27
S_5 (B_5)	1	0.266	23
S_6 (B_6)	2	0.129	11
S_7 (B_7)	4	0.071	6

CROSS REACTIVITY

PROSTAGLANDIN E_2	100.0%
PROSTAGLANDIN B_1	63.0%
PROSTAGLANDIN E_3	52.0%
PROSTAGLANDIN E_1	50.0%
PROSTAGLANDIN B_2	2.65%
6-KETO-PROSTAGLANDIN E_1	0.91%
PROSTAGLANDIN A_1	0.78%
PROSTAGLANDIN A_2	0.30%
PROSTAGLANDIN $F_{1\alpha}$	0.13%
PROSTAGLANDIN $F_{2\alpha}$	0.06%
LEUKOTRIENE B_4	0.02%
PROSTAGLANDIN D_2	0.01%
6-KETO-PROSTAGLANDIN $F_{1\alpha}$	0.01%
13,14-DIHYDRO-15-KETO-PROSTAGLANDIN $F_{2\alpha}$	0.01%
TETRANOR PGEM	<0.01%

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TECHNICAL ASSISTANCE

Technical assistance is available Monday-Friday, between 8:00 a.m. and 6:00 p.m. EST.

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D406510-07/20/09