GE Healthcare

Amersham Prostaglandin E_2 Biotrak Enzymeimmunoassay (EIA) System

Product Booklet

Codes: RPN222 (96 wells)
RPN22210 (960 wells)

Page finder

1. Legal

http//www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK

2. Handling

2.1. Safety warnings and precautions

Warning: ***For research use only.*** Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin and eyes. In the case of contact with skin or eyes, wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.

Note: The assay protocol may require the use of Sulfuric acid, Indomethacin and Sodium Hydroxide.

Warning: Sulfuric acid is corrosive, Indomethacin is toxic and Sodium Hydroxide is caustic.

Please follow the manufacturers' safety data sheets relating to the safe handling and use of these materials.

2.2. Storage

Store at -15°C to -30°C.

2.3. Expiry

The expiry date is stated on the package and will be at least 4 weeks from the date of despatch.

3. Components

RPN222 contains 1x each component listed. RPN22210 contains 10 x each component listed but WITHOUT lysis reagents 1 and 2.

Microplate: The plate contains 12 x 8 well strips coated with sheep anti-mouse IgG. Ready for use.

Assay buffer: Bottle contains 50 ml of Phosphate buffer concentrate with Kathon which on dilution gives a 0.1 M Phosphate buffer pH 7.5 containing 0.9%(w/v) Bovine Serum Albumin and 0.5%(w/v) Kathon.

Standard: Bottle contains Prostaglandin E_2 at a concentration of 256 ng/ml in Ethanol. Ready for use

Antibody: Bottle contains lyophilised Prostaglandin E_2 antibody.

Prostaglandin E_2 conjugate: Bottle contains lyophilised Prostaglandin E_2 conjugated to Horseradish Peroxidase.

Wash buffer: Bottle contains 12.5 ml of Phosphate buffer concentrate which on dilution gives 0.01 M Phosphate buffer pH 7.5 containing 0.05%(v/v) Tween™ 20.

TMB substrate: Bottle contains 3,3',5,5' Tetramethylbenzidine (TMB)/Hydrogen Peroxide. Ready for use.

Lysis reagent 1: Dodecyltrimethylammonium Bromide, 2 g, solid.

Lysis reagent 2: Solid, 5 g. The lysis reagent 2 vial contains no chemicals classified as hazardous.

4. Critical parameters

The following points are critical:

When carrying out PGE_2 enzyme immunoassays, please take particular note of the following instructions regarding critical steps:

- It is essential to read the complete instruction booklet before starting work.
- Washing the plate
  Thoroughly wash the plate before adding the **substrate**. This can be done either manually or automatically provided that the following points are noted:

Manual plate washing

- Use a wash bottle.
- **Completely fill** each well with wash buffer.
- **Completely empty** each well between washes.
- After the final wash it is **essential** that all wells are emptied. Tap the plate briskly on a pad of tissues to effect this.

Automatic plate washing

- Ensure that all wells are filled and emptied **completely** with each cycle.
- Uneven washing will cause poor results. If the effectiveness of your instrument is in doubt, wash manually as described above.
- There is no difference in results when using either automated or hand washing procedures, if the instrument is carefully maintained.
- To aid efficient washing in automated plate washers, the strips should be levelled with the edge of the microplate lid before each washing stage.

- Allow samples and all reagents to reach room temperature prior to performing the assay.
- Mix samples and all reagents thoroughly before use.
- Avoid excessive foaming of reagents.
- Avoid handling the tops of the wells both before and after filling.
- Keep the wells covered with lids except when adding reagents and reading.
- Standards and samples should be assayed in duplicate.
- Run a separate standard curve for each microplate.
- Carry out a microscopic evaluation, with 0.4% Trypan blue, before and after lysing cells.

5. Other materials required

The following materials and equipment are required but not supplied:

- Pipettes or pipetting equipment with disposable polypropylene tips (50 µl, 100 µl, 500 µl, 1 ml and 5 ml)
- Disposable polypropylene test tubes
- Vortex mixer
- Refrigerator
- Glass measuring cylinders (50 ml, 100 ml, 500 ml)
- Distilled or deionised water
- Spectrophotometric plate reader capable of measuring at 450 nm
- 1.0 M Sulfuric acid
- Microplate shaker
- Magnetic stirrer and stirrer bars
- 0.4% Trypan blue solution
- Centrifuge and microplate holders for centrifuge (if using suspension cells).
- Automatic plate washer (optional)

6. Description

The Prostaglandin E_2 (PGE_2) competitive Biotrak™ enzymeimmunoassay system from GE Healthcare is specifically designed for research purposes. RPN222 includes protocols using novel lysis reagents in order to facilitate simple and rapid extraction of PGE_2 from cell cultures and plasma samples. These components obviate the need for removal of extracting reagents prior to measurement, ensuring PGE_2 is directly available for subsequent analysis. With the method for measurement of PGE_2 in plasma, complex, time-consuming sample preparation procedures are avoided.

Each pack of RPN222 contains sufficient material for 96 wells. This allows the construction of one standard curve and the measurement of 37 unknowns in duplicate. Each pack of RPN22210 contains sufficient material for 960 wells. This allows for the construction of one standard curve and the measurement of 37 unknowns in duplicate per plate.

- Elimination of inconvenient, time-consuming extraction procedures
- Flexible method-choice of assay protocols
- Direct measurement of PGE_2 in plasma
- Rapid room temperature assay ~2 hours
- Dual range 2.5–320 pg/well (20–25°C protocol)
- Dual range 1.0–32 pg/well (2–8°C protocol)
- Non-radioactive
- Specific for PGE_2
- Precise and accurate measurement
- Ready to use substrate
- Colour coded reagents

Lysis reagent 1 hydrolyses cell membranes to release intracellular PGE_2, or, dissociates PGE_2 from soluble receptors and interfering binding proteins present in plasma. Lysis reagent 2 sequesters

the key component in lysis reagent 1 and ensures PGE_2 is free for subsequent analysis. The detergent/sequestrant complex does not interfere with antigen: antibody binding. Lysis reagent 1 is simply added to cultured cells or plasma samples, followed by a ten minute incubation before assay (figure 1). The antisera and PGE_2 Peroxidase conjugate are reconstituted with lysis reagent 2. The assay is based on competition between unlabelled PGE2 and a fixed quantity of Peroxidase-labelled PGE_2 for a limited number of binding sites on a PGE2 specific antibody (figure 2).

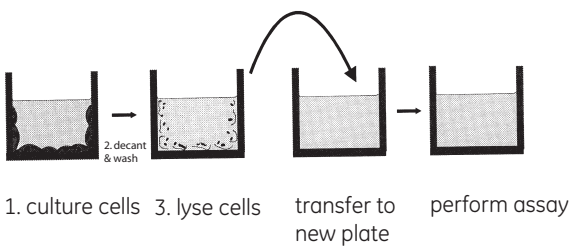

Figure 1. Cell lysis - protocol 3 intracellular method

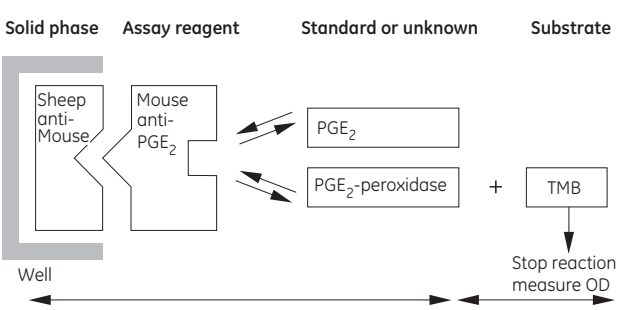

Figure 2. EIA principle

7. Protocol Selection

The assay procedure can be carried out in one of five ways using the RPN222 kit.
The assay procedure can be carried out in one of two ways using either protocol 1 or 2 for the RPN22210 kit.

Protocol 1. PGE_2 may be measured in the range 2.5–320 pg/well (50–6400 pg/ml) using an enzymeimmunoassay procedure (for the measurement of PGE_2 in Urine and cell culture media without extraction, or for measurement of PGE_2 in tissues and cultures, with traditional sample extraction methods such as solvent and solid-phase methods). This protocol is carried out at room temperature and is the standard assay procedure.

Protocol 2. Higher sensitivity may be obtained by performing the assay at 2–8°C. Using this approach, standard curves ranging from 1–32 pg/well (20–640 pg/ml) are obtained. This protocol is not subject to routine quality control.

Protocol 3. This method describes a direct, intracellular method for the measurement of PGE_2 in tissue culture lysates where cells are grown in flasks, vessels or on plates. Cells are lysed for 10 minutes (with the reagents provided in the kit), and an aliquot is transferred to a second plate for assay. This method is particularly useful for monitoring actual cell responsiveness to a drug, stimulant or cyclooxygenase inhibitors.

PGE_2 is measured in the range 2.5–320 pg/well (50–6400 pg/ml).

Protocol 4. This method also uses the lysis reagents. Here the combined amount of intracellular and cell supernatant PGE_2 is measured. This fraction is referred to as 'total' cellular PGE_2 and has the additional benefit of not requiring decantation of the cell culture supernatant. It measures the total responsiveness of a cell to a given stimulus. PGE_2 is measured in the range 2.5–320 pg/well (50–6400 pg/ml).

Protocol 5. A direct procedure for estimating PGE_2 in plasma. Here lysis reagent 1 is simply added to plasma samples and aliquots removed for subsequent analysis, thus obviating the need for tedious sample extraction procedures. PGE_2 is measured in the range 2.5–320 pg/well (50–6400 pg/ml).

Table 1. Summary of protocols

Curve range	Urine samples/cell culture supernatants, no sample extraction needed	Tissue samples. Sample extraction required (eg Amprep)	Tissue samples where higher sensitivity is needed. Sample extraction required (eg Amprep)	Cell cultures Lysis reagents and protocols provided	Plasma samples No sample extraction required
2.5–320 pg/well	Protocol 8.1 (See page 14)	Protocol 8.1 (See page 14)		Protocol 8.3 Intracellular PGE_2 (See page 27) Protocol 8.4 'Total' cellular PGE_2 (See page 35)	Protocol 8.5 (See page 40)
1.32 pg/well			Protocol 8.2 (See page 21)		

8. Protocols

8.1. Standard EIA Procedure

(Curve range 2.5–320 pg/well; 50–6400 pg/ml, for measurement of PGE_2 in urine, tissues and cell culture supernatants)

8.1.1. Specimen collection, sample preparation and purification

- Samples should be processed immediately after collection and assayed as soon as possible. Prostaglandin E_2 has been reported to be stable in urine if kept frozen.
- Several methods are available for purifying PGE_2. These include both solvent (1–2) and solid-phase (3–4) extraction
- Useful information on prostaglandin immunoassays may be found in three scientific papers (5–7).
- An effective method for sample purification of PGE_2 uses Amprep™. However, it remains the responsibility of the investigator to validate the chosen extraction procedure.

1. Pipette 0.5 ml of sample (homogenised tissue etc) into a 1.5 ml polypropylene microcentrifuge tube.
2. Add 0.5 ml of a 1:4 water:Ethanol solution.
3. Add 10 µl of glacial Acetic acid.
4. Mix gently and leave at room temperature for 5 minutes.
5. Centrifuge at 2500 X g for approximately 2 minutes.
6. Prime a 100 mg Amprep C18 minicolumn with 2 column volumes of 10% Ethanol.
7. Remove the supernatant from the centrifuge tube and apply to the Amprep column.
8. Wash the column with 1 volume of distilled water
9. Wash the column with 1 volume of Hexane.

10. Elute the PGE_2 with 2 X 0.75 ml of Ethyl Acetate.

11. Collect the Ethyl Acetate fractions and evaporate to dryness under Nitrogen.

8.1.2. EIA Procedure

Reagent preparation

Note: All reagents must be allowed to equilibrate to room temperature prior to use. Either distilled or deionised water may be used for reagent preparation. The microplate and enzyme substrate are supplied ready for use when equilibrated to room temperature.

Assay buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.

2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

Prostaglandin E_2 conjugate

1. Add 6 ml diluted assay buffer and replace the stopper.

2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.

3. Take 2 ml of the reconstituted conjugate and add to 4 ml of diluted assay buffer.

Antibody

1. Add 6 ml diluted assay buffer and replace the stopper.

2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.

3. Take 3 ml of the diluted antibody and add to 3 ml of diluted assay buffer.

Wash buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

The standard, reconstituted antibody and Peroxidase conjugate should be stored at -15°C to -30°C and re-used within two weeks. The assay buffer, wash buffer, unused microtitre wells and substrate may be stored at 2–8°C and re-used within seven days.

Preparation of working standards
Note: It is important to use a clean pipette tip for each dilution. Standards should be used within 1 hour of preparation.

1. Pipette 3.9 ml diluted assay buffer into a suitable polypropylene container.
2. Add 100 µl of stock standard and mix thoroughly. This provides the top standard of 320 pg/50 µl.
3. Label 7 polypropylene tubes 2.5, 5, 10, 20, 40, 80 and 160 pg.
4. Pipette 500 µl diluted assay buffer into these tubes.
5. Transfer 500 µl of the 320 pg standard into the 160 pg tube and mix thoroughly.
6. Repeat the doubling dilution successively with the remaining tubes.
7. 50 µl aliquots from each of the serial dilutions will give rise to 8 standard levels ranging from 2.5 to 320 pg/well.

Assay method
Note: It is important that all reagents are equilibrated to room temperature before use.

1. Prepare the reagents and working standards as described in the 'reagent preparation' section.

2. Set up the microplate with sufficient wells for running of all blanks, standards and samples in duplicate (see figure 3).
3. Pipette 100 µl of diluted assay buffer into the non-specific binding (NSB) wells.
4. Pipette 50 µl of diluted assay buffer into the zero standard (0) wells.
5. Starting with the most dilute pipette 50 µl of each standard into the appropriate wells.
6. Pipette 50 µl of each unknown sample into the appropriate wells.
7. Pipette 50 µl of diluted antibody into all wells except the blank (B) and NSB. **REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 15).
8. Pipette 50 µl of diluted conjugate into all wells except the blank. **REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 15).
9. Cover the plate with the lid provided.
10. Incubate the plate at room temperature (20–25°C) for 1 hour on a microplate shaker.
11. Aspirate and wash all wells 4 times with wash buffer ensuring that all wells are completely filled and emptied at each wash.
12. Blot the plate on tissue ensuring any residual buffer is removed. Thorough washing is essential for good performance.
13. Immediately pipette 150 µl of room temperature equilibrated enzyme substrate into all wells.
14. Cover the plate with the lid provided and mix on a microplate shaker for exactly 30 minutes at room temperature (15–30°C).
15. The blue colour that develops may be read at 630 nm. However it is recommended that the reaction is halted prior to end point determination by the addition of 100 µl of 1 M Sulfuric acid to all

wells. The optical density can be read at 450 nm within 30 minutes.

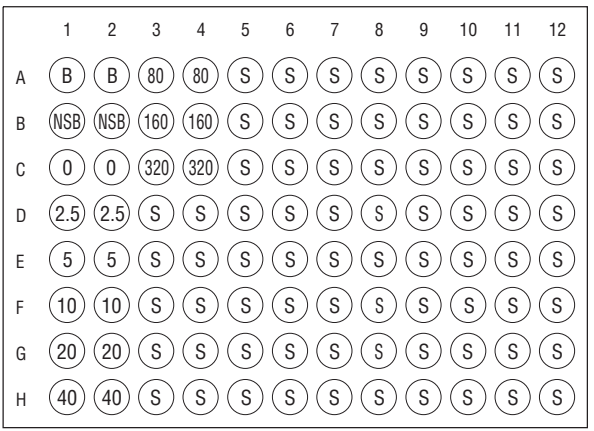

Figure 3. Recommended positioning of standard (2.5–320 pg/ well) and sample (S) well

8.1.3. Data Processing

Calculation of results

The calculation is illustrated using representative data and is the same for all protocols.

The assay data should be similar to that shown in table 2.

1. Calculate the average optical density (OD) for each set of replicate wells.
2. Calculate the percent bound for each standard and sample using the following relationship:

$$\%B/B_0 = \frac{(\text{standard or sample OD-NSB OD}) \times 100}{(\text{zero standard OD-NSB OD})}$$

A standard curve may be generated by plotting the percent B/B_0 as a function of the log prostaglandin E_2 concentration. Plot $\%B/B_0$ (*y* axis) against pg prostaglandin E_2 standard per well (x axis). The curve shape should be similar to figure 4, if plotted on semi-log paper. The pg/well value of samples can be read directly from the graph. Figure 4 shows a plot of the data from table 2.

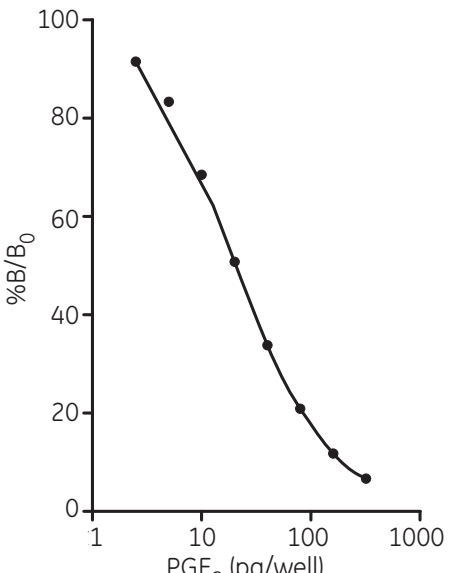

Figure 4. Prostaglandin E_2 curve, protocol 1 - Standard EIA procedure

Typical assay data

Table 2. Typical assay data, protocol 1

Standard (pg/well)	Optical density	Mean OD at 450 nm	Mean OD-NSB	%B/B_0
Blank	0.042 0.042	0.042		
NSB	0.050 0.051	0.050		
0	1.862 1.881	1.872	1.822	
2.5	1.733 1.700	1.717	1.667	91.5
5	1.563 1.570	1.566	1.516	83.3
10	1.310 1.284	1.297	1.247	68.5
20	0.973 0.978	0.975	0.925	50.8
40	0.665 0.668	0.666	0.616	33.8
80	0.422 0.441	0.432	0.382	20.9
160	0.278 0.254	0.266	0.216	11.8
320	0.173 0.172	0.172	0.122	6.7

8.2. High sensitivity EIA procedure

(Curve range 1–32 pg/well; 20–640 pg/ml for tissue samples where high sensitivity is required)

8.2.1. Specimen collection and sample purification

Details on extracting PGE_2 from tissue samples may be found on page 15.

8.2.2. EIA Procedure

Reagent preparation

Note: All reagents must be allowed to equilibrate to room temperature prior to use. Either distilled or deionised water may be used for reagent preparation. The microplate and enzyme substrate are supplied ready for use when equilibrated to room temperature.

Assay buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

Prostaglandin E_2 conjugate

1. Add 6 ml diluted assay buffer and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided. No further dilution is required but the reconstituted conjugate should be kept at 2–8°C for the duration of the assay.

Antibody

1. Add 6 ml diluted assay buffer and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided. **No further dilution is required.**

Wash buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

The standard, reconstituted antibody and Peroxidase conjugate should be stored at -15°C to -30°C and re-used within two weeks. The assay buffer, wash buffer, unused microtitre wells and substrate may be stored at 2–8°C and re-used within seven days.

Preparation of working standards

Note: It is important to use a clean pipette tip for each dilution. Standards should be used within 1 hour of preparation.

1. Label 7 polypropylene tubes 1, 2, 4, 8 ,16, 32 and 640 pg.
2. Into the 32 and 640 pg tubes, pipette 1.9 ml of diluted assay buffer.
3. Into the remaining tubes pipette 500 µl of diluted assay buffer.
4. Pipette 100 µl of the stock standard into the 640 pg tube and mix thoroughly. **THIS STANDARD DILUTION IS NOT INCLUDED IN THE ASSAY.**
5. Transfer 100 µl from the 640 pg tube to the 32 pg tube and mix thoroughly.
6. Transfer 500 µl from the 32 pg tube to the 16 pg tube and mix thoroughly.
7. Repeat this doubling dilution with the remaining tubes.

Assay method

Note: It is important that all reagents are equilibrated to room temperature before use.

1. Prepare the reagents and working standards as described in the 'reagent preparation' section.

2. Set up the microplate with sufficient wells for running of all blanks, standards and samples in duplicate (see figure 5).
3. Pipette 100 µl of diluted assay buffer into the non-specific binding (NSB) wells.
4. Pipette 50 µl of diluted assay buffer into the zero standard (0) wells.
5. Starting with the most dilute pipette 50 µl of each standard into the appropriate wells.
6. Pipette 50 µl of each unknown sample into the appropriate wells.
7. Pipette 50 µl of diluted antibody into all wells except the blank (B) and NSB.
8. Cover the plate with the lid provided and incubate on ice, in a refrigerator or cold room at 2–8°C for 3 hours. Ensure that the reconstituted conjugate is placed with the plate.
9. At the end of the 3 hour incubation, add 50 µl of conjugate to all wells except the blank. Ensure that the plate is kept on ice during the procedure.
10. Cover the plate with the lid provided and incubate on ice, in a refrigerator or cold room at 2–8°C for 1 hour.
11. Aspirate and wash all wells 4 times with wash buffer ensuring that all wells are completely filled and emptied at each wash.
12. Blot the plate on tissue ensuring any residual buffer is removed. Thorough washing is essential for good performance.
13. Immediately pipette 150 µl of room temperature equilibrated enzyme substrate into all wells.
14. Cover the plate with the lid provided and mix on a microplate shaker for exactly 30 minutes at room temperature (15–30°C).
15. The blue colour that develops may be read at 630 nm. However it is recommended that the reaction is halted prior to end point

determination by the addition of 100 µl of 1 M Sulfuric acid to all wells. The optical density can be read at 450 nm within 30 minutes.

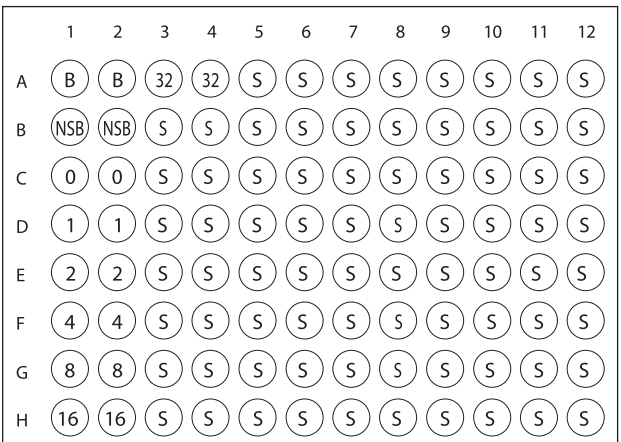

Figure 5. Recommended positioning of standard (1–32 pg/well) and sample (S) wells

8.2.3. Data Processing

Calculation of results

The method for calculating results is shown on page 18. The assay data should be similar to that shown in table 3. Figure 6 shows a plot of the data from table 3.

Typical assay data

Table 3. Typical assay data (protocol 2 - high sensitivity EIA procedure)

Standard (pg/well)	Optical density	Mean OD at 450 nm	Mean OD-NSB	%B/B_0
Blank	0.066 0.066	0.066		
NSB	0.067 0.067	0.067		
0	1.104 1.126	1.115	1.048	
1	1.053 1.028	1.042	0.975	93
2	0.889 0.908	0.899	0.832	79
4	0.696 0.681	0.689	0.622	59
8	0.424 0.395	0.410	0.343	33
16	0.213 0.246	0.230	0.163	15
32	0.145 0.151	0.148	0.081	8

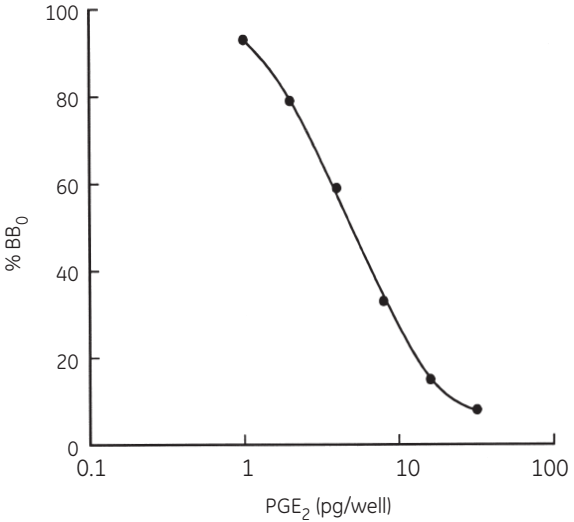

Figure 6. Prostaglandin E_2 standard curve, protocol 2 - high sensitivity

8.3. Intracellular PGE_2 measurement using novel lysis reagents

(Curve range 2.5–320 pg/well; 50–6400 pg/ml, for the measurement of PGE_2 in cell cultures only)

Note: This procedure can be followed with RPN22210

8.3.1. EIA Procedure

Reagent preparation

Note: All reagents must be allowed to equilibrate to room temperature prior to use. Either distilled or deionised water may be used for reagent preparation. The microplate and enzyme substrate are supplied ready for use when equilibrated to room temperature.

Assay buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

NOTE: lysis reagents 1 and 2 (solids) require 30 minutes mixing at room temperature to dissolve in assay buffer. This is readily achieved using a beaker and magnetic stirrer.

Lysis reagent 1

1. Transfer the contents of the bottle (lysis reagent 1, solid) into a beaker using repeated washing with 60 ml of diluted assay buffer. Use continuous stirring throughout. Ensure the contents of the bottle are completely dissolved.
2. Adjust the final volume to 80 ml with assay buffer and mix thoroughly. The final solution contains 2.5% Dodecytrimethyl Ammonium Bromide. This is buffer A.

3. Take 10 ml of buffer A and make up to 100 ml with diluted assay buffer to give a final 0.25% solution of Dodecyltrimethyl Ammonium Bromide. Mix thoroughly. This is lysis reagent 1 (working solution).

Lysis reagent 2

1. Transfer the contents of the bottle (lysis reagent 2, solid) into a beaker using repeated washing with 80 ml of diluted assay buffer. Use continuous stirring throughout. Ensure the contents of the bottle are completely dissolved.
2. Adjust the final volume to 100 ml with assay buffer and mix thoroughly. The final solution contains 5% lysis reagent 2. This is buffer B.
3. Take 10 ml of buffer B and carefully make up to 33.3 ml in a 50 ml measuring cylinder with diluted assay buffer to give a final 1.5% solution of lysis reagent 2. Mix thoroughly. This is lysis reagent 2 (working solution).

Prostaglandin E_2 conjugate

1. Add 6 ml diluted lysis reagent 2 (working solution) and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Take 2 ml of the reconstituted conjugate and add to 4 ml of diluted lysis reagent 2 (working solution).

Antibody

1. Add 6 ml diluted lysis reagent 2 (working solution) and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.

3. Take 4 ml of the diluted antibody and add to 2 ml of diluted lysis reagent 2 (working solution).

Wash buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

The standard, reconstituted antibody and Peroxidase conjugate should be stored at -15°C to -30°C and re-used within two weeks. The assay buffer, wash buffer, unused microtitre wells and substrate may be stored at 2–8°C and re-used within seven days.

Preparation of working standards
Note: It is important to use a clean pipette tip for each dilution. Standards should be used within 1 hour of preparation.

1. Pipette 3.9 ml diluted lysis reagent 1 (working solution) into a suitable polypropylene container.
2. Add 100 µl of stock standard and mix thoroughly. This provides the top standard of 320 pg/50 µl.
3. Label 7 polypropylene tubes 2.5, 5, 10, 20, 40, 80 and 160 pg.
4. Pipette 500 µl diluted lysis reagent 1 (working solution) into these tubes.
5. Transfer 500 µl of the 320 pg standard into the 160 pg tube and mix thoroughly.
6. Repeat the doubling dilution successively with the remaining tubes.
7. 50 µl aliquots from each of the serial dilutions will give rise to 8 standard levels ranging from 2.5 to 320 pg/well.

Note: Care should be taken when preparing working standards. Dodecyltrimethylammonium Bromide may cause frothing. Vigorous pipetting should be avoided.

Cell lysis methods

Adherent cells

1. Culture cells (100 µl) in standard 96-well microplates (tissue culture grade), with cell concentrations between 10^4–10^6 cells/well.
2. Incubate plate overnight at 37°C (5% CO_2 and 95% humidity). **Note: do not use cell cultures that are over-confluent (e.g. at 10^7 cells/ml) as cells may be lost during decantation.**
3. Add 100 µl of drug, agonist etc. Incubate for suitable time period.
4. Decant or aspirate excess culture media and wash the cells thoroughly with PBS. This step is readily carried out by immersing the plate into a tray containing 1 litre of PBS. Gently decant or aspirate the PBS. **Note: Carry out a microscopic check to ensure the cells have not been lost during the washing procedure.**
5. Add 100 µl/well of diluted lysis reagent 1 (working solution).
6. Agitate cells after lysis reagent 1 is added. This is to facilitate cell lysis and can be achieved by vigorous, successive pipetting. Incubate cells for 10 minutes after adding the lysis reagent.
7. Carry out a microscopic evaluation using Trypan blue to check cells have lysed. Cell membranes may still be visible after cell lysis. Lysed cells are now ready for use in the enzymeimmunoassay protocol. Immediately process the extracted PGE_2 for measurement with the enzymeimmunoassay (see 'assay protocol').
8. Transfer 50 µl aliquots of cell lysate to the sheep anti-mouse Ig coated plate for assay.

Suspension cells

Note: If suspension cells are used, special microplates are needed for the centrifugation step.

1. Culture cells (100 µl) in standard 96-well microplates (tissue

culture grade), with cell concentrations between 10^4–10^6 cells/well.

2. Incubate plate overnight at 37°C (5% CO_2 and 95% humidity).
3. Add 100 µl of drug, agonist etc. Incubate for suitable time period.
4. Centrifuge the microplate using a centrifugal microplate adapter (1500–2000 X g) for 3 minutes to form a pellet in each well.
5. Decant or aspirate excess culture media and wash the cells thoroughly with PBS. This step is readily carried out by immersing the plate into a tray containing 1 litre of PBS. Gently decant or aspirate the PBS. **Note: Carry out a microscopic check to ensure the cells have not been lost during the washing procedure.**
6. Add 100 µl/well of diluted lysis reagent 1 (working solution).
7. Agitate cells after lysis reagent 1 is added. This is to facilitate cell lysis and can be achieved by vigorous, successive pipetting. Incubate cells for 10 minutes after adding the lysis reagent.
8. Carry out a microscopic evaluation using Trypan blue to check cells have lysed. Cell membranes may still be visible after cell lysis. Lysed cells are now ready for use in the enzymeimmunoassay protocol. Immediately process the extracted PGE_2 for measurement with the enzymeimmunoassay (see 'assay protocol').
9. Transfer 50 µl aliquots of cell lysate to the sheep anti-mouse Ig coated plate for assay.

Assay method

Note: It is important that all reagents are equilibrated to room temperature before use.

1. Prepare the reagents and working standards as described in the 'reagent preparation' section.
2. Set up the microplate with sufficient wells for running of all blanks, standards and samples in duplicate (see figure 3, page 18).

3. Pipette 50 µl of lysis reagent 1 and 50 µl of lysis reagent 2 (working solutions) into the NSB wells.
4. Pipette 50 µl of lysis reagent 1 (working solution) into the zero standard (0) wells.
5. Starting with the most dilute, pipette 50 µl of each standard into the appropriate wells.
6. Pipette 50 µl of each unknown sample into the appropriate wells (see previous section for sample preparation).
7. Pipette 50 µl of diluted antibody into all wells except the blank and the NSB. **Note: REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 27)
8. Pipette 50 µl of diluted conjugate into all wells except the blank. **Note: REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 27)
9. Cover the plate with the lid provided.
10. Incubate the plate at room temperature (20–25°C) for 1 hour on a microplate shaker.
11. Aspirate and wash all wells 4 times with wash buffer ensuring that all wells are completely filled and emptied at each wash.
12. Blot the plate on tissue ensuring any residual buffer is removed. Thorough washing is essential for good performance.
13. Immediately pipette 150 µl of room temperature equilibrated enzyme substrate into all wells.
14. Cover the plate with the lid provided and mix on a microplate shaker for exactly 30 minutes at room temperature (15–30°C).
15. The blue colour that develops may be read at 630 nm. However it is recommended that the reaction is halted prior to end point determination by the addition of 100 µl of 1 M Sulfuric acid to all wells. The optical density can be read at 450 nm within 30 minutes.

8.3.2. Data Processing

Calculation of results

The method for calculating results is shown on page 18. Typical results are shown in table 4. Figure 7 shows a plot from table 4.

Typical assay data

Table 4. Typical assay data for the intra-, 'total' cellular and plasma assays (protocols 8.3, 8.4 and 8.5).

Standard (pg/well)	Optical density	Mean OD at 450 nm	Mean OD-NSB	%B/B_0
Blank	0.049 0.050	0.050		
NSB	0.060 0.061	0.061		
0	1.229 1.235	1.232	1.171	
2.5	1.186 1.181	1.184	1.123	96
5	1.160 1.154	1.157	1.096	94
10	1.073 1.075	1.074	1.013	87
20	0.946 0.952	0.949	0.888	76
40	0.774 0.775	0.775	0.714	61
80	0.575 0.584	0.580	0.519	44
160	0.396 0.398	0.397	0.336	29
320	0.275 0.278	0.277	0.216	18

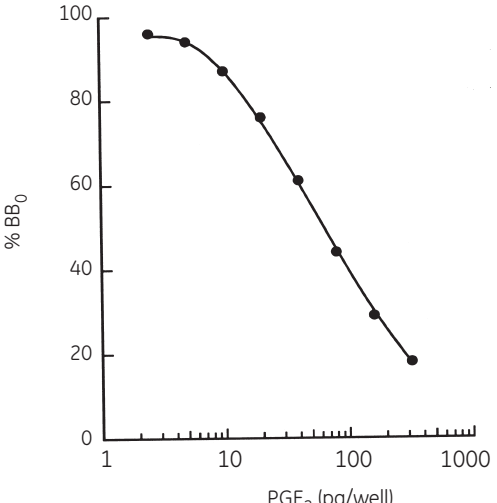

Figure 7. Typical standard curve for intra-, total cellular and plasma assays (protocols 3, 4 and 5)

8.4. Total cellular PGE_2 measurement using novel lysis reagents

(Curve range 2.5–320 pg/well; 50–6400 pg/ml, for the measurement of PGE_2 in cell cultures only)

8.4.1. EIA Procedure

Reagent preparation

Note: All reagents must be allowed to equilibrate to room temperature prior to use. Either distilled or deionised water may be used for reagent preparation. The microplate and enzyme substrate are supplied ready for use when equilibrated to room temperature.

Assay buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.

2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

NOTE: lysis reagents 1 and 2 (solids) require 30 minutes mixing at room temperature to dissolve in assay buffer. This is readily achieved using a beaker and magnetic stirrer.

Lysis reagent 1

1. Transfer the contents of the bottle (lysis reagent 1, solid) into a beaker using repeated washing with 60 ml of diluted assay buffer. Use continuous stirring throughout. Ensure the contents of the bottle are completely dissolved.

2. Adjust the final volume to 80 ml with assay buffer and mix thoroughly. The final solution contains 2.5% Dodecytrimethyl Ammonium Bromide. This is buffer A which is used in the cell lysis method in the 'total' cellular PGE_2 assay.

3. Take 10 ml of buffer A and make up to 100 ml with diluted assay buffer to give a final 0.25% solution of Dodecyltrimethyl Ammonium Bromide. Mix thoroughly. This is lysis reagent

1 (working solution), and is used for the preparation of standards.

Lysis reagent 2

1. Transfer the contents of the bottle (lysis reagent 2, solid) into a beaker using repeated washing with 80 ml of diluted assay buffer. Use continuous stirring throughout. Ensure the contents of the bottle are completely dissolved
2. Adjust the final volume to 100 ml with assay buffer and mix thoroughly. The final solution contains 5% lysis reagent 2. This is buffer B.
3. Take 10 ml of buffer B and carefully make up to 33.3 ml in a 50 ml measuring cylinder with diluted assay buffer to give a final 1.5% solution of lysis reagent 2. Mix thoroughly. This is lysis reagent 2 (working solution).

Prostaglandin E_2 conjugate

1. Add 6 ml diluted lysis reagent 2 (working solution) and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Take 2 ml of the reconstituted conjugate and add to 4 ml of diluted lysis reagent 2 (working solution).

Antibody

1. Add 6 ml diluted lysis reagent 2 (working solution) and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Take 4 ml of the diluted antibody and add to 2 ml of diluted lysis reagent 2 (working solution).

Wash buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

The standard, reconstituted antibody and Peroxidase conjugate should be stored at -15°C to -30°C and re-used within two weeks. The assay buffer, wash buffer, unused microtitre wells and substrate may be stored at 2–8°C and re-used within seven days.

Preparation of working standards

Note: It is important to use a clean pipette tip for each dilution. Standards should be used within 1 hour of preparation.

1. Pipette 3.9 ml diluted lysis reagent 1 (working solution) into a suitable polypropylene container.
2. Add 100 µl of stock standard and mix thoroughly. This provides the top standard of 320 pg/50 µl.
3. Label 7 polypropylene tubes 2.5, 5, 10, 20, 40, 80 and 160 pg.
4. Pipette 500 µl diluted lysis reagent 1 (working solution) into these tubes.
5. Transfer 500 µl of the 320 pg standard into the 160 pg tube and mix thoroughly.
6. Repeat the doubling dilution successively with the remaining tubes.
7. 50 µl aliquots from each of the serial dilutions will give rise to 8 standard levels ranging from 2.5 to 320 pg/well.

Note: Care should be taken when preparing working standards. Dodecyltrimethylammonium Bromide may cause frothing. Vigorous pipetting should be avoided.

Cell lysis method-adherent and suspension cells

1. Culture cells (160 µl volumes) in standard 96-well microplates (tissue-culture grade), with cell concentrations between 10^4 and 10^6 cells/well.

2. Incubate the plate overnight at 37°C (5% CO_2 and 95% humidity).

3. Stimulate cells with (e.g. 20 µl) agonist, cell stimulant, inhibitor as required. Do not decant or aspirate the cell culture media. Incubate agonist/cell stimulant with cultures depending on the required experimental conditions.

4. Add 20 µl of buffer A (2.5% Dodecyltrimethylammonium Bromide in assay buffer). The final volume in the wells should be 200 µl, each containing 0.25% buffer A (final concentration) and agonist/ stimulant which is equivalent to the lysis reagent 1 working solution.

5. Following the addition of buffer A, agitate cells to facilitate cell lysis. This can be achieved by vigorous, successive pipetting. Incubate the plate for 10 minutes at room temperature.

6. Carry out a microscopic evaluation with Trypan blue to check the cells have lysed. Cell membranes may still be visible after cell lysis. Lysed cells are now ready for use in the enzymeimmunoassay protocol. Immediately process the extracted PGE_2 for measurement with the enzymeimmunoassay (see 'assay protocol').

7. Transfer 50 µl aliquots of cell lysate on to the sheep anti-mouse Ig coated plate for assay.

Assay method

Note: It is important that all reagents are equilibrated to room temperature before use.

1. Prepare the reagents and working standards as described in the 'reagent preparation' section.

2. Set up the microplate with sufficient wells for running of all blanks, standards and samples in duplicate (see figure 3, page 18).

3. Pipette 50 µl of lysis reagent 1 and 50 µl of lysis reagent 2 (working solutions) into the NSB wells.

4. Pipette 50 µl of lysis reagent 1 (working solution) into the zero standard (0) wells.

5. Starting with the most dilute, pipette 50 µl of each standard into the appropriate wells.

6. Pipette 50 µl of each unknown sample into the appropriate wells (see previous section for sample preparation).

7. Pipette 50 µl of diluted antibody into all wells except the blank and the NSB. **Note: REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 35)

8. Pipette 50 µl of diluted conjugate into all wells except the blank. **Note: REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 35)

9. Cover the plate with the lid provided.

10. Incubate the plate at room temperature (20–25°C) for 1 hour on a microplate shaker.

11. Aspirate and wash all wells 4 times with wash buffer ensuring that all wells are completely filled and emptied at each wash.

12. Blot the plate on tissue ensuring any residual buffer is removed. Thorough washing is essential for good performance.

13. Immediately pipette 150 µl of room temperature equilibrated enzyme substrate into all wells.

14. Cover the plate with the lid provided and mix on a microplate shaker for exactly 30 minutes at room temperature (15–30°C).

15. The blue colour that develops may be read at 630 nm. However it is recommended that the reaction is halted prior to end point

determination by the addition of 100 µl of 1 M Sulfuric acid to all wells. The optical density can be read at 450 nm within 30 minutes.

8.4.2. Data Processing

For typical data and calculation of results see pages 33–34.

8.5. Measurement of PGE_2 in plasma using novel lysis reagents

(Curve range 2.5–320 pg/well; 50–6400 pg/ml)

8.5.1. Specimen collection and sample purification

Specimen collection

All samples should be processed immediately after collection and assayed as soon as possible. Levels of PGE_2 in plasma decrease significantly if stored at -15°C to -30°C for one week.

- EDTA or Citrate are recommended as anticoagulants as Heparin can lead to activation of white blood cells.

Anticoagulant preparation

1. Dispense 2 g of Disodium EDTA and 0.8 g NaCl in distilled water.
2. Adjust to pH 7.4 with 1 M NaOH.
3. Make up to 100 ml with distilled water.
4. Dissolve 50 mg Indomethacin in 3.5 ml of absolute Ethanol.
5. For each collection tube, mix 0.25 ml of the EDTA solution and 0.05 ml of the Indomethacin.
6. The EDTA solution must be stirred while the Indomethacin is added.

Blood collection

1. Using the above anticoagulant (or equivalent) collect 10 ml of blood into a tube.
2. Centrifuge the blood immediately.

3. Remove the upper plasma layer and rapidly freeze.
4. Store at -15°C to -30°C.

8.5.2. EIA Procedure

Reagent preparation

Note: All reagents must be allowed to equilibrate to room temperature prior to use. Either distilled or deionised water may be used for reagent preparation. The microplate and enzyme substrate are supplied ready for use when equilibrated to room temperature.

Assay buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.
2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

NOTE: lysis reagents 1 and 2 (solids) require 30 minutes mixing at room temperature to dissolve in assay buffer. This is readily achieved using a beaker and magnetic stirrer.

Lysis reagent 1

1. Transfer the contents of the bottle (lysis reagent 1, solid) into a beaker using repeated washing with 60 ml of diluted assay buffer. Use continuous stirring throughout. Ensure the contents of the bottle are completely dissolved.
2. Adjust the final volume to 80 ml with assay buffer and mix thoroughly. The final solution contains 2.5% Dodecytrimethylammonium Bromide. This is buffer A and is used for the preparation of plasma samples.
3. Take 10 ml of buffer A and make up to 100 ml with diluted assay buffer to give a final 0.25% solution of Dodecyltrimethylammonium Bromide. Mix thoroughly. This is lysis reagent 1 (working solution) and is used for the preparationof standards.

Lysis reagent 2

1. Transfer the contents of the bottle (lysis reagent 2, solid) into a beaker using repeated washing with 80 ml of diluted assay buffer. Use continuous stirring throughout. Ensure the contents of the bottle are completely dissolved.
2. Adjust the final volume to 100 ml with assay buffer and mix thoroughly. The final solution contains 5% lysis reagent 2. This is buffer B.
3. Take 10 ml of buffer B and carefully make up to 33.3 ml in a 50 ml measuring cylinder with diluted assay buffer to give a final 1.5% solution of lysis reagent 2. Mix thoroughly. This is lysis reagent 2 (working solution).

Prostaglandin E_2 conjugate

1. Add 6 ml diluted lysis reagent 2 (working solution) and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Take 2 ml of the reconstituted conjugate and add to 4 ml of diluted lysis reagent 2 (working solution).

Antibody

1. Add 6 ml diluted lysis reagent 2 (working solution) and replace the stopper.
2. Gently mix until the contents are completely dissolved. Vigorous agitation and foaming should be avoided.
3. Take 4 ml of the diluted antibody and add to 2 ml of diluted lysis reagent 2 (working solution).

Wash buffer

1. Transfer the contents of the bottle to a 500 ml cylinder by repeated washing with distilled water.

2. Adjust the final volume to 500 ml with distilled water and mix thoroughly.

The standard, reconstituted antibody and Peroxidase conjugate should be stored at -15°C to -30°C and re-used within two weeks. The assay buffer, wash buffer, unused microtitre wells and substrate may be stored at 2–8°C and re-used within seven days.

Preparation of working standards
Note: It is important to use a clean pipette tip for each dilution. Standards should be used within 1 hour of preparation.

1. Pipette 3.9 ml diluted lysis reagent 1 (working solution) into a suitable polypropylene container.
2. Add 100 µl of stock standard and mix thoroughly. This provides the top standard of 320 pg/50 µl.
3. Label 7 polypropylene tubes 2.5, 5, 10, 20, 40, 80 and 160 pg.
4. Pipette 500 µl diluted lysis reagent 1 (working solution) into these tubes.
5. Transfer 500 µl of the 320 pg standard into the 160 pg tube and mix thoroughly.
6. Repeat the doubling dilution successively with the remaining tubes.
7. 50 µl aliquots from each of the serial dilutions will give rise to 8 standard levels ranging from 2.5 to 320 pg/well.

Note: Care should be taken when preparing working standards. Dodecyltrimethylammonium Bromide may cause frothing. Vigorous pipetting should be avoided.

Preparation of plasma samples
Measurements should be made in plasma not serum. Blood must be collected as described on page 40.

1. Take 900 µl of plasma sample and add 100 µl of buffer A. Mix well.
2. Immediately process the sample for measurement with the enzymeimmunoassay (see 'assay protocol').
3. Transfer 50 µl aliquots of sample on to the sheep anti-mouse Ig coated plate for assay.

Assay method

Note: It is important that all reagents are equilibrated to room temperature before use.

1. Prepare the reagents and working standards as described in the 'reagent preparation' section.
2. Set up the microplate with sufficient wells for the running of all blanks, standards and samples in duplicate (see figure 3, page 18).
3. Pipette 50 µl of lysis reagent 1 and 50 µl of lysis reagent 2 (working solutions) into the NSB wells.
4. Pipette 50 µl of lysis reagent 1 (working solution) into the zero standard (0) wells.
5. Starting with the most dilute, pipette 50 µl of each standard into the appropriate wells.
6. Pipette 50 µl of each unknown sample into the appropriate wells (see previous section for sample preparation).
7. Pipette 50 µl of diluted antibody into all wells except the blank and the NSB. **Note: REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 40)
8. Pipette 50 µl of diluted conjugate into all wells except the blank. **Note: REMEMBER THE EXTRA DILUTION OF THIS COMPONENT** (see page 40)
9. Cover the plate with the lid provided.

10. Incubate the plate at room temperature (20–25°C) for 1 hour on a microplate shaker.

11. Aspirate and wash all wells 4 times with wash buffer ensuring that all wells are completely filled and emptied at each wash. Blot the plate on tissue ensuring any residual buffer is removed. **Thorough washing is essential for good performance.**

12. Immediately pipette 150 µl of room temperature equilibrated enzyme substrate into all wells.

13. Cover the plate with the lid provided and mix on a microplate shaker for exactly 30 minutes at room temperature (15–30°C).

14. The blue colour that develops may be read at 630 nm. However it is recommended that the reaction is halted prior to end point determination by the addition of 100 µl of 1 M Sulfuric acid to all wells. The optical density can be read at 450 nm within 30 minutes.

8.5.3. Data Processing

For typical data and calculation of results see page 33.

For the recovery of known concentrations of PGE_2 from plasma, see technical application examples on pages 48–51.

9. Additional Information

9.1. Specificity

The cross-reactivity, as determined by the concentration giving 50% B/B_0, with a number of related compounds is shown in tables 5 and 6 below:

Table 5. Cross-reactivity, standard assay (protocol 8.1)

Compound	% Cross-reactivity
PGE_1	21
$PGF_{2\alpha}$	0.121
6-Keto-$PGF_{1\alpha}$	0.110
Arachidonic acid	<0.0001

Table 6. Cross-reactivity for high sensitivity assay (protocol 8.2)

Compound	% Cross-reactivity
PGE_1	17
PGD_2	0.04
PGA_2	0.077
PGB_2	2.57
$PGF_{2\alpha}$	5.68
$PGF_{1\alpha}$	0.1
6-Keto-$PGF_{1\alpha}$	1.67
13,14-Dihydro-15-Keto-PGE_2	<0.1
13,14-Dihydro-15-Keto-$PGF_{2\alpha}$	<0.1
5_α, 7_α-Dihydroxy-11-Keto-tetranorprostane-1,16-dioic acid	<0.1
15-Keto-$PGF_{2\alpha}$	<0.1
TXB_2	<0.1

9.2. Reproducibility

Within-assay precision

The within assay precision was calculated by measuring controls in the assay. The results are shown in table 7 below:

Table 7.

	Protocol 8.1			Protocol 8.2		
Control	**Mean**	**% CV**	**n**	**Mean**	**% CV**	**n**
A	10.2	10.9	10	–	–	–
B	20.4	7.6	12	2.89	8.1	12
C	43.11	7.8	12	6.27	7.5	12

Between-assay precision

The between-assay precision was assessed by repeated measurement of the same control in successive assays. The results are shown in table 8 below:-

Table 8.

	Protocol 8.1			Protocol 8.2		
Control	**Mean**	**% CV**	**n**	**Mean**	**% CV**	**n**
A	14.0	18.4	10	–	–	–
B	27.9	11.1	10	4.7	10.6	16
C	77.0	9.0	9	12.8	10.6	16

9.3. Sensitivity

The sensitivity, defined as the amount of prostaglandin E_2 needed to reduce zero dose binding by two standard deviations is 2 pg/well (equivalent to 40 pg/ml) for protocol 8.1.

The sensitivity, defined as above, is 0.8 pg/well (equivalent to 16 pg/ml) for protocol 8.2.

9.4. Technical applications and examples

Example 1. Measurement of intracellular and supernatant PGE_2 in calcium ionophore stimulated mouse 3T3 cells using protocols 1 and 3.

Table 9.

	Calcium ionophore A23187 concentration (µM)		
	0	**100**	**% increase**
Intracellular PGE_2 (pg/ml)	51	3288	6347
Supernatant PGE_2 (pg/ml)	5664	11 376	100

Cells were stimulated with A23187 for five minutes and PGE_2 in the intracellular and supernatant fractions measured. Zero ionophore values were resting cells prepared in the absence of A23187. The results show significant levels of PGE_2 are secreted into the culture media in the absence of stimulation. When A23187 is added, there is a doubling in measured values in the culture supernatant. However, intracellular levels increase by over 6000 percent.

Example 2. Effect of aspirin on intracellular and supernatant PGE_2 generation from mouse 3T3 cells using protocols 8.1 and 8.3.

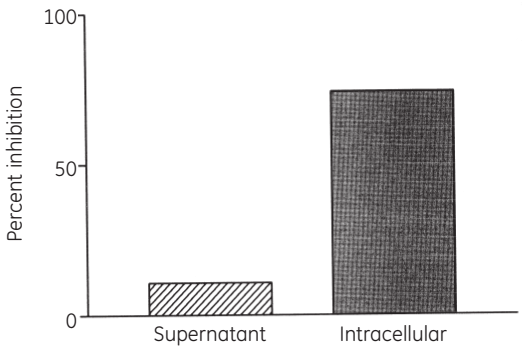

Figure 8.

Mouse 3T3 cells were treated overnight with 4 µm aspirin (a cyclooxygenase inhibitor) before stimulating for 5 minutes with the Calcium ionophore A23187. Intracellular and cell culture supernatant PGE_2 were measured with the EIA. There was a 11% inhibition of PGE_2 levels in the culture supernatant and a 74% inhibition in intracellular values.

Example 3. Effect of Indomethacin on intracellular and supernatant PGE_2 generation from mouse 3T3 cells using protocol 1 and 3.

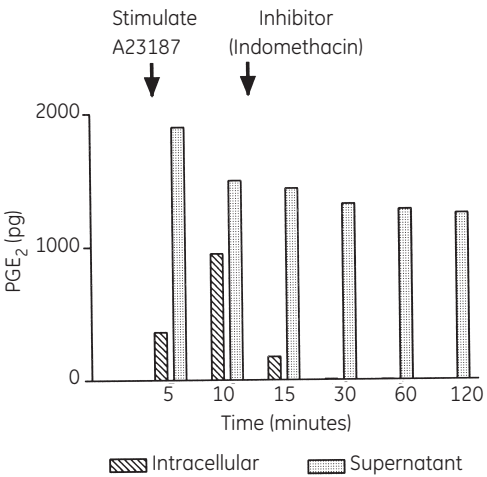

Figure 9.

Mouse 3T3 cells were stimulated with the Calcium Ionophore A23187 before addition of Indomethacin (a cyclooxygenase inhibitor). Intracellular and cell culture supernatant PGE_2 were measured with the EIA over a two hour period. Intracellular PGE_2 exhibited a significant reduction on addition of the inhibitor, but there was only a marginal decrease in levels measured in the culture supernatant.

Example 4. Recovery of PGE_2 from normal human plasma using protocol 5

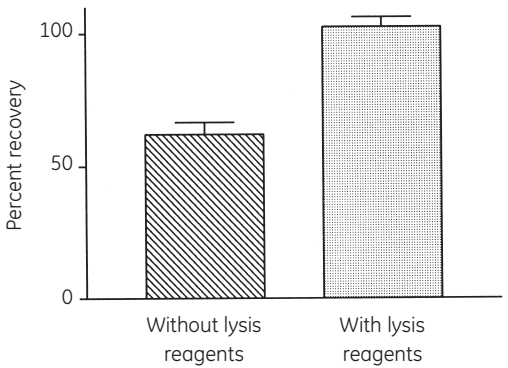

Figure 10.

Normal human plasma was spiked with known concentrations of PGE_2 and assayed with and without lysis reagents. Mean recovery of PGE_2 was ~55% in the absence of lysis reagents. Recovery of added PGE_2 rose to 100% in the presence of the lysis reagents.

10. Troubleshooting guide

Problems	Remedies
1. Low optical densities	**1.** Check plate reader wavelength. **2.** Check incubation time and temperature. **3.** Check that reagents are equilibrated to room temperature before use. **4.** Check that the kit reagents have been stored correctly.
2. High optical densities	**1.** Ensure that every well is completely filled and emptied at every wash step. **2.** Ensure that automatic washers are functioning correctly. **3.** Check incubation times and temperatures. **4.** Ensure that plates have been blotted on tissue paper after washing.
3. Flat curves/poor reproducibility	**1.** Check pipette calibration. **2.** Check preparation of working standards. **3.** Ensure troughs used with multichannel pipettes are separate and dedicated to individual components. **4.** Ensure that washing procedures have been carried out correctly.

11. References

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12. Related products

Thromboxane B_2, [^{125}I]	RIA	RPA516
Leukotriene B_4	EIA	RPN223
Leukotriene C_4/D_4/E_4	EIA	RPN224
Prostaglandin D_2, [^{3}H]	RIA	TRK890
Prostaglandin E_2, [^{125}I]	RIA/AM	RPA530
6-Keto-prostaglandin $F_{1\alpha}$	EIA	RPN221
6-Keto-prostaglandin $F_{1\alpha}$ [^{125}I]	RIA/AM	RPA515
Thromboxane B_2	EIA	RPN220

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