

GE Healthcare

Amersham
Prostaglandin E₂ [¹²⁵I]
Biotrak Assay System
with Magnetic Separation

100 assay tubes

Product Booklet

Code: RPA530



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1. Legal

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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.

Caution: Radioactive material. Instructions relating to the handling, use, storage and disposal of radioactive materials.

1. Upon receipt, vials or ampoules containing radioactive material should be checked for contamination. All radioactive materials should be stored in specially designated areas and suitable shielding should be used where appropriate. Access to these areas should be restricted to authorised personnel only.
2. Radioactive material should be used by responsible persons only in authorised areas. Care should be taken

to prevent ingestion or contact with skin or clothing. Protective clothing, such as laboratory overalls, safety glasses and gloves should be worn whenever radioactive materials are handled. Where this is appropriate, the operator should wear personal dosimeters to measure radiation dose to the body and fingers.

3. No smoking, drinking or eating should be allowed in areas where radioactive materials are used. Avoid actions that could lead to the ingestion of radioactive materials, such as the pipetting of radioactive solutions by mouth.
4. Vials containing radioactive materials should not be touched by hand; wear suitable protective gloves as normal practice. Use forceps when handling vials containing 'hard' beta emitters such as phosphorus-32 or

gamma emitting labelled compounds. Ampoules likely to contain volatile radioactive compounds should be opened only in a well ventilated fume cabinet.

5. Work should be carried out on a surface covered with absorbent materials or in enamel trays of sufficient capacity to contain any spillage. Working areas should be monitored regularly.
6. Any spills of radioactive material should be cleaned immediately and all contaminated materials should be decontaminated or disposed of as radioactive waste via an authorised route. Contaminated surfaces should be washed with a suitable detergent to remove traces of radioactivity.
7. After use, all unused radioactive materials should be stored in specifically designated areas. Any radioactive product not required or any materials that have come into contact

with radioactivity should be disposed of as radioactive waste via an authorised route.

8. Hands should be washed after using radioactive materials. Hands and clothing should be monitored before leaving the designated area, using appropriate instruments to ensure that no contamination has occurred. If radioactive contamination is detected, hands should be washed again and rechecked. Any contamination persisting on hands and clothing should be reported to the responsible person so that suitable remedial actions can be taken.
9. Certain national/international organisations and agencies consider it appropriate to have additional controls during pregnancy. Users should check local regulations.

Most countries have legislation governing the handling, use, storage, disposal and transportation of radioactive

materials. The instructions set out above complement local regulations or codes of practice. Such regulations may require that a person be nominated to oversee radiological protection. Users of radioactive products must make themselves aware of and observe the local regulations or codes of practice which relate to such matters.

Warning: Contains sodium azide. See safety data sheet on pages 30.

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 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 or eyes. In the case of contact with skin or eyes wash immediately with water (see

safety data sheet for specific advice).

Note that the protocol requires the use of ethyl acetate, glacial acetic acid, NaOH, hexane and ethanol.

Warning: Ethyl acetate and ethanol are highly flammable, acetic acid is flammable and corrosive, hexane is flammable and harmful and sodium hydroxide is corrosive. Please follow the manufacturers' safety data sheets for the safe handling and use of these materials.

2.2. Storage

Store at 2–8°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 of the assay system

The pack contains the following assay components, sufficient material for 100 tubes. All components for this kit should be stored at 2–8°C.

Standard

Prostaglandin E₂ standard (methyl oximated) containing 5% (w/v) lactose, 8 ng, lyophilised.

Tracer

[¹²⁵I] Prostaglandin E₂ proline-tyrosine conjugate (methyl oximate derivative), containing 5% (w/v) lactose, ~55.5kBq, ~1.5 µCi, lyophilised.

Antiserum

Antiserum specific for the methyl oximate derivative, containing 5% (w/v) lactose, lyophilised.

Assay buffer

Assay buffer concentrate, 10 ml. On dilution to 100 ml this assay buffer consists of 0.05 M Tris/HCl buffer pH 7.4 containing 0.9% NaCl, 0.01% Triton X-100, 0.1% (w/v) bactogelatin and preservative.

Methyl oximation reagent

A ready to use solution of methoxyamine hydrochloride and sodium acetate in water:ethanol (9:1 v/v), pH 5.6, 11 ml. This reagent is used to convert samples to their methyl oximate derivative prior to assay.

Amerlex™-M second antibody reagent

Donkey anti-rabbit serum coated on to magnetisable polymer particles, colour coded blue-green, with sodium azide, 30 ml ready for use. See safety data sheet on pages 30.

4. Description

- Sensitive assay ~0.8 pg/tube
- Highly specific antiserum
- Flexible protocol, 2 hours at 25°C or overnight at room temperature
- Rapid assay, can be completed within 3-4 hours
- Convenient Amerlex-M separation
- Working range 1.25 to 160 pg
- Simple one column sample purification

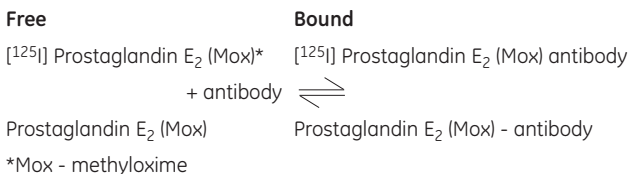
The Biotrak™ prostaglandin E₂ [¹²⁵I]assay system from GE Healthcare has been specifically designed for research purposes. It combines the use of a high specific activity prostaglandin E₂ tracer, an antiserum specific for prostaglandin E₂ (raised against the methyl oximate derivative) and a prostaglandin E₂ methyl oximated standard. This provides a rapid, simple and sensitive method for the determination of prostaglandin E₂ *in vitro* in the range 1.25 to 160 pg/tube.

Each pack contains sufficient material for 100 assay tubes. This permits the construction of one standard curve and the assay of 39 unknowns in duplicate. Alternatively, the reconstituted reagents can be stored at 2–8°C for up to 14 days, allowing construction of standard curves on separate occasions.

The assay is based on the competition between unlabelled prostaglandin E₂ (methyl oximate derivative) and a fixed quantity of ¹²⁵I-labelled prostaglandin E₂ (methyl oximate derivative) for a limited number of binding sites on a prostaglandin E₂ specific antibody which is specific for the methyl oximate derivative. The amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand. Measurement of antibody-bound radioactivity enables the amount of unlabelled prostaglandin E₂ in the sample to be determined.

Separation of the antibody-bound prostaglandin E₂ from unbound ligand is achieved by adsorption of the bound fraction using the Amerlex-M reagent. This contains a donkey anti-rabbit second antibody bound to magnetisable polymer particles. Separation can be effected either by application of a magnetic field or centrifugation, followed by decantation of the supernatant. Measurement of radioactivity in the pellet quantitates the amount of radioactive ligand bound by the antibody. The concentration of unlabelled prostaglandin E₂ is then determined by interpolation from a standard curve. The basis of the assay is illustrated in figure 1.

Figure 1.



4.1. Principle of the assay

The assay is based on the conversion of prostaglandin E₂ to the methyl oximate derivative by methoxyamine hydrochloride. The assay then utilises the competition between unlabelled methyl oximated prostaglandin E₂ and a fixed quantity of ¹²⁵I-labelled prostaglandin E₂ (methyl oximate derivative) for a specific antibody raised against methyl oximated prostaglandin E₂. The advantages of using this approach are

- prostaglandin E₂ is more stable as its methyl oximate form, therefore extracted samples once derivatised can be stored for longer periods without significant decomposition occurring;
- increased specificity and sensitivity, as the antiserum has been raised against methyl oximated prostaglandin E₂.

5. Critical parameters

The following points are critical:

- Working standards should be freshly prepared before each assay, and not re-used.
- In view of the instability of prostaglandin E_2 in plasma it is recommended that samples should be processed, purified and derivatised to the methyl oximate derivative as quickly as possible and stored at -15°C to -30°C in their derivatised form
- Particular care should be taken when reconstituting the standard.

6. Additional materials and equipment required

The following materials and equipment are required but not supplied pipettes or pipetting equipment with disposable polypropylene tips (100 µl, 200 µl, 250 µl, 500 µl).

- Vortex mixer (an Amerlex-M multivortexer is available from Johnson & Johnson)
- Glass measuring cylinder (100 ml)
- 250 ml glass beaker
- Disposable polypropylene or polystyrene tubes (12 x 75 mm) (Disposable polypropylene tubes are supplied by Sarstedt International, Rommelsdore, 5223 Numbrecht, Germany)
- Distilled or deionised water
- Gamma scintillation counter
- Incubation or constant temperature water bath capable of holding water temperature at 25°C
- Amerlex-M separators comprising a magnetic base and assay rack, are available from Johnson & Johnson. These are for use in the magnetic separation protocol.

Note: For sample purification, Amprep™ C18 minicolumns (code RPN 1900) are required.

Note: For the centrifugal protocol, centrifugation and decantation racks plus refrigerated centrifuge capable of at least 1500 x g, will be required. For sample preparation centrifuge capable of 2500 x g will be required.

7. Specimen collection and sample preparation

Prostaglandin E_2 is present in a wide variety of biological material. It is unlikely that a single preparation procedure will prove suitable for all samples. This section provides information about sample purification procedures which have been reported in literature. The information is provided for guidance only. It remains the investigators responsibility to validate the chosen sample preparation procedure.

7.1. Specimen collection

It is recommended that all samples be processed immediately after collection and assayed as soon as possible. Although prostaglandin E_2 is reported to be stable in urine if kept frozen the level of prostaglandin E_2 in plasma decreases significantly if stored at -15°C to -30°C for one week.

Note: In view of the instability of prostaglandin E_2 in plasma it is recommended that samples should be processed, purified and derivatised to the methyl oximate derivative as quickly as possible and stored at -15°C to -30°C in their derivatised form. Samples are stable for up to 6 days under these conditions.

Collect the blood in a tube with an anticoagulant, centrifuge the blood immediately and rapidly freeze the plasma. If blood samples cannot be rapidly processed it is recommended that indomethacin or aspirin is added to the anticoagulant. Either of these compounds will inhibit the subsequent metabolism of arachidonic acid to prostaglandins. One effective method is to collect the blood in a tube containing EDTA and indomethacin. Mix 0.95 ml of an EDTA solution (2 g disodium EDTA and 0.8 g NaCl adjusted to pH 7.4 with NaOH and made to a final volume of 100 ml in distilled water) with 0.05 ml of 0.04 M indomethacin solution (50 mg indomethacin dissolved in

3.5 ml absolute ethanol) for the treatment of 10 ml blood. The EDTA solution must be mixed while the indomethacin is being added or the indomethacin will precipitate. Store the plasma samples at -15°C to -30°C until the assay is conducted.

7.2. Sample purification

Information describing prostaglandin radioimmunoassays is contained in three relevant articles (13-15). It is well established that non-esterified fatty acids can interfere with prostaglandin assays (16-18). Several methods are available for purifying PGE₂. A number of investigators have reported useful solvent extraction procedures (19-20). Solid phase extraction procedures such as the one described by Powell (21) have been reported to be the methods of choice.

One effective method using Amprep minicolumns has been reported by Kelly (22) and is given below:

Into a 1.5 ml polypropylene microcentrifuge tube pipette 0.5 ml of plasma sample, 0.5 ml of 1:4 water:ethanol and 10 µl of glacial acetic acid. Gently mix and leave at room temperature for 5 minutes. Centrifuge at 2500 x g for ~2 minutes. Remove the supernatant and apply to an Amprep C18 (100 mg size, product code RPN 1900) minicolumn which has been primed with 2 column volumes of 10% ethanol.

Wash the column with 1 column volume of distilled water and 1 column volume of hexane. Elute the prostaglandin E₂ with 2 x 0.75 ml ethyl acetate. Collect the ethyl acetate fractions and evaporate to dryness under nitrogen. Using this method, levels of prostaglandin E₂ in normal plasma have been reported at ~5 pg/ml. This value agrees with previously reported levels of prostaglandin E₂ in plasma between 3-15 pg/ml (12,14).

7.3. Methyl oximation procedure

Prior to analysing extracted samples using this assay system it is necessary to convert extracted prostaglandin E₂ into its methyl oximate derivative using the methyl oximation reagent provided. This is performed as follows:

1. Reconstitute the dried prostaglandin E₂ fraction from the Amprep purification protocol given above with 100 µl of phosphate buffered gelatin saline pH 7.0.
2. To the reconstituted sample add 100 µl of the methyl oximation reagent. Vortex mix the resulting solution and incubate at 60°C for 1 hour to allow methyl oximation of the sample to take place.

Note: Alternatively derivatisation can be achieved using an overnight incubation at room temperature. Similar results have been obtained using this approach.

3. Following methyl oximation, dilute to a final volume of 500 µl with the phosphate buffered gelatin saline. Samples prepared in this way can be assayed directly or can be stored at -15°C to -30°C for up to 6 days before analysis.

8. Radioimmunoassay procedure

8.1. Reagent preparation

Assay buffer

Either distilled or deionised water may be used to dilute the assay buffer. The 10 ml buffer concentrate is semi-liquid when removed from the refrigerator. Mixing for 5 to 10 minutes on a magnetic stirrer at room temperature is sufficient to liquify the gel. If necessary gently warming to about 30°C will facilitate the process. Transfer the vial contents with washings to a 250 ml glass beaker. Make up the buffer to a total volume of 100 ml and stir for a further 5 minutes.

Tracer

Reconstitute the contents of the vial with 11 ml of assay buffer. Mix thoroughly.

Antiserum

Reconstitute the contents of the vial with 11 ml of assay buffer. Mix thoroughly.

Standard

Note: The prostaglandin E₂ standard provided is in the methyl oximate form, no further derivatisation of this solution is required. Reconstitute the contents of the standard vial with 2.5 ml of assay buffer.

Briefly vortex mix and allow the vial contents to equilibrate to room temperature. The vial contains 3200 pg/ml of prostaglandin E₂ and serves as the stock solution to prepare a series of standards as follows. Particular care should be taken during this procedure.

8.2. Preparation of working standards

1. Label 8 polypropylene or polystyrene tubes 160 pg, 80 pg, 40 pg, 20 pg, 10 pg, 5 pg, 2.5 pg and 1.25 pg. Pipette 500 μ l of assay buffer into each tube.
2. Pipette 500 μ l of the stock solution into the tube marked 160 pg and vortex mix thoroughly.
3. Transfer 500 μ l from the tube marked 160 pg into the tube marked 80 pg and vortex mix thoroughly.
4. Repeat this doubling dilution successively with the remaining tubes.
5. 100 μ l aliquots of each serial dilution give rise to standard levels of prostaglandin E₂ ranging from 1.25 to 160 pg per tube.

Note: Working standards should be freshly prepared before each assay, and not re-used. The stock standard should be recapped and returned to the refrigerator.

8.3. Assay protocol

The procedure is summarised in table 1.

1. Label polypropylene tubes (12 x 75 mm) in duplicate for total counts (TC), non-specific binding (NSB), zero standard (B₀) standards and samples.
2. Starting with the most dilute, pipette 100 μ l of standard into the appropriately labelled tubes. Use a new pipette tip for each standard.
3. Pipette 100 μ l of sample into appropriately labelled tubes. Use a new pipette tip for each standard.
4. Briefly vortex mix the tracer vial, pipette 100 μ l of tracer into each vial.
5. Briefly vortex mix the antiserum vial, pipette 100 μ l of antiserum into all tubes except the NSB and TC.

6. Pipette 200 μ l of assay buffer into the NSB and TC tubes, and 100 μ l of assay buffer into the B₀ tubes.
7. Vortex mix all tubes and incubate for 2 hours at 25°C in a water bath (see note page 19).
8. Gently shake and swirl the bottle containing Amerlex-M second antibody reagent (blue-green) to ensure a homogeneous suspension. Add 250 μ l into each tube except the TC. Incubate at room temperature for 15 minutes.
9. Separate the antibody bound fraction using either magnetic separation or centrifugation as described below.

Magnetic separation

Remove TC tubes and attach the rack to the Amerlex-M separator base and ensure that all tubes are in contact with the base plate. Leave for 15 minutes. After separation, **do not remove the rack** from the separator base. Pour off and discard the supernatant. **Keeping the separator inverted**, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

Centrifugation

Centrifuge all tubes at 4°C for 10 minutes at 1500 x g or greater. After centrifugation, place the tubes carefully into suitable decantation racks, then pour off and discard the supernatant. Keeping the tubes inverted, place the tubes on a pad of absorbent tissues and allow to drain for 5 minutes.

10. On completion of either magnetic or centrifugal separation, firmly blot the rims of the inverted tubes on the tissue pad to remove any adhering liquid. Do not re-invert the tubes once they have been turned upright.
11. Determine the radioactivity present in each tube by counting for at least 60 seconds in a gamma scintillation counter.

Note: If preferred an alternative procedure using an overnight

incubation at room temperature may be used. Standard curve parameters are similar to those obtained with the 2 hour incubation.

Table 1. Radioimmunoassay protocol (All volumes are in microlitres)

Tube	Total count (TC)	Non-specific binding (INSB)	Zero standard (B ₀)	Standards	Samples
Standards	-	-	-	100	-
Samples	-	-	-	-	100
Tracer	100	100	100	100	100
Antiserum	-	-	100	100	100
Buffer	200	200	100	-	-
Vortex mix, and incubate for 2 hours at 25°C in a water bath					
Amerlex-M	-	250	250	250	250

Vortex mix, incubate for 15 minutes at room temperature. Separate using either magnetic separation for 15 minutes or by centrifuging for 10 minutes at 1500 x g. Decant supernatants, drain for 5 minutes and count.

8.4. Re-use of kit components

Reconstituted components should be stored at 2–8°C and may be reused within 14 days of dilution.

9. Data processing

9.1. Calculation of results

Taking into account the 60 day half-life of the iodine tracer the assay data collected should be similar to the data shown in table 2.

1. Calculate the average counts per minute (cpm) for each set of replicate tubes
2. Calculate the normalised percent bound for each standard and sample using the following relationship:

$$\%B/B_0 = \frac{(\text{Standard or sample cpm} - \text{NSB cpm}) \times 100}{(B_0 \text{ cpm} - \text{NSB cpm})}$$

A standard curve may be generated by plotting the normalised percent bound as a function of the $\log_{10}$ prostaglandin E_2 concentration.

Plot $\% B/B_0$ (y axis) against pg standard per tube (x axis) as shown in figure 2. The pg/tube value of the samples can then be read directly from the graph.

9.2 Typical assay data

Table 2. Typical assay data

Tube	cpm	%B/B ₀
Total count	23814	
	24005	
NSB	1046	
	1093	
Zero standard	11643	100
	11426	
Standard 1.25 pg/tube	10740	93
	10900	
Standard 2.5 pg/tube	9848	84
	9891	
Standard 5.0 pg/tube	9124	77
	9111	
Standard 10 pg/tube	7631	63
	7601	
Standard 20 pg/tube	6049	48
	6127	
Standard 40 pg/tube	4588	34
	4686	
Standard 80 pg/tube	3513	23
	3453	
Standard 160 pg/tube	2372	13
	2441	

The counts will decline with the age of the tracer

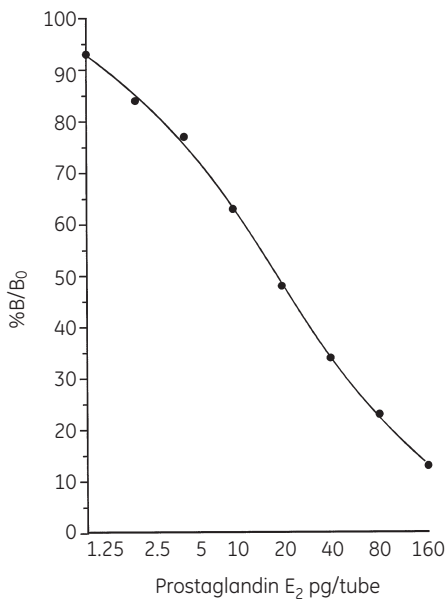


Figure 2. Prostaglandin E₂ standard curve

10. Additional information

10.1. Specificity

The specificity data for the Prostaglandin E₂ antiserum are as shown below:

Prostaglandins	% Cross-reactivity (50% B/B ₀ displacement)
Prostaglandin E ₂	100
Prostaglandin E ₁	~5
8-Iso prostaglandin E ₂	<5
20(OH) Prostaglandin E ₂	<3
19(OH) Prostaglandin E ₂	<0.01
15-Keto prostaglandin E ₂	<0.01
13,14-Dihydro-15-keto-prostaglandin E	<0.01
Prostaglandin D ₂	<0.001
Prostaglandin E _{2α}	<0.001
6-Keto prostaglandin E ₁	<0.001
13,14 Dihydro-15-keto prostaglandin F	<0.001
6-Keto prostaglandin F _{1α}	<0.001
Arachidonic acid	<0.001

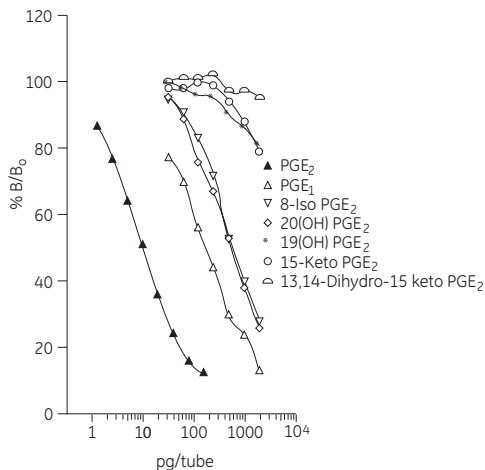


Figure 3a. Cross reactivity profile

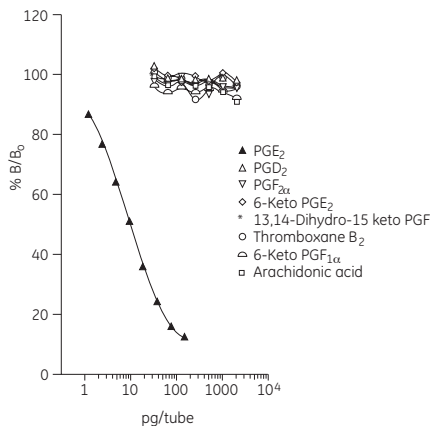


Figure 3b. Cross reactivity profile

10.2. Sensitivity

1.0 pg/tube. Defined as the amount of prostaglandin E₂ needed to reduce the zero dose binding by two standard deviations.

10.3. Precision

1. Within-assay precision

Control	Measured value (pg/tube)	CV%	Number of replicates
Low	4.31±0.22	5.1	10
Medium	11.26±0.42	3.7	10
High	32.42±1.95	5.9	9

2. Between-assay precision

Controls	Measured value (pg/tube)	CV%	Number of replicates
Low	4.32±0.43	10	8
Medium	13.15±1.27	9.7	8
High	38.12±4.1	11.0	8

3. Precision profile

Data derived from 19 assays

Standard (pg/tube)	%B/B ₀ ±SD	%CV
1.25	90.5 ± 5.9	6.5
2.5	81.9 ± 3.0	3.7
5	69.7 ± 3.5	5.0
10	54.4 ± 3.5	6.4
20	39.8 ± 2.6	6.5
40	27.3 ± 1.8	6.6
80	17.3 ± 1.8	10.4
160	10.4 ± 1.5	14.4

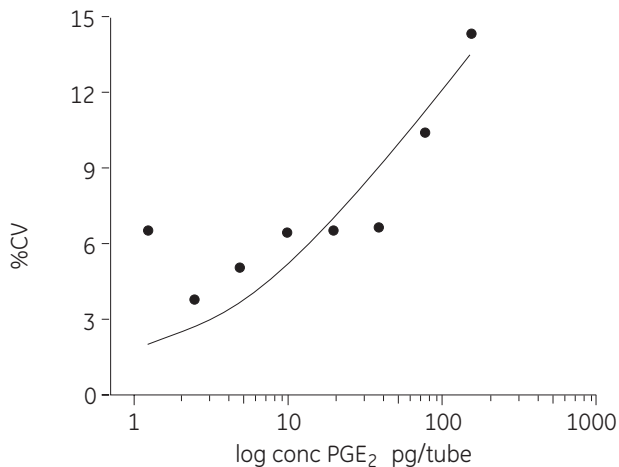


Figure 4. Precision profile

10.4. Assay drift

One standard curve and 80 control tubes were performed in one assay. Medium controls were assayed immediately after the standard curve (positions 1 to 10) and at the end of the assay (positions 71 to 80).

Medium control position number	Measured value (pg/tube)	%CV
1 to 10	12.6 ± 1.1	6.1
71 to 80	12.35 ± 1.35	7.1

10.5. Amerlex-M accessories

Amerlex-M separator (comprising 50 tube rack and magnetic base)

Amerlex-M multivortexer

Amerlex-M racks (4 in a pack)

These products are available from Johnson & Johnson plc, or their assigned distributors.

10.6. Background and references

A diverse array of mammalian cells and tissues enzymatically oxidise arachidonic acid to physiologically active compounds. These compounds include thromboxanes, prostacyclin, prostaglandins and leukotrienes.

Arachidonate metabolism in the cyclooxygenase pathway leads initially to the formation of prostaglandin H_2 (1–3).

Prostaglandin E_2 is formed from prostaglandin H_2 by the action of a specific isomerase (4).

As reviewed (5), prostaglandin E_2 is the predominant arachidonate metabolite from the kidney of many species. It is thought to participate in the modulation of renin release along with

prostaglandin I₂ (6). Prostaglandin E₂ is also produced by macrophages and may mediate some effects of macrophages on neighbouring cells as well as influencing the functional state of the macrophage itself (5, 7, 8). The possible participation of prostaglandin E₂ and other cyclooxygenase products in a variety of other physiological processes has also been recently reviewed (9–11).

The availability of GE Healthcare's prostaglandin E₂ radioimmunoassay system will facilitate the evaluation of the physiological role of this important arachidonic acid metabolite.

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10.7. Related products

Biotrak eicosanoid assay range

Thromboxane B ₂	EIA	RPN 220
Thromboxane B ₂ [¹²⁵ I]	RIA	RPA 516
Leukotriene B ₄	EIA	RPN 223
Leukotriene B ₄ [³ H]	RIA	TRK 940
Leukotriene C ₄ specific (Mab), [³ H]	RIA	TRK 905

Leukotriene C ₄ /D ₄ /E ₄	EIA	RPN 224
Platelet activating factor (PAF), [³ H]	SPA	TRK 990
Prostaglandin E ₂	EIA	RPN 222
6-Keto-prostaglandin F _{1α}	EIA	RPN 221
6-Keto-prostaglandin F _{1α} , [¹²⁵ I]	RIA/AM	RPA 515
Prostaglandin F _{2α} , [³ H]	RIA	TRK 900

Amprep range

Amprep C18 minicolumns	RPN 1900
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Safety data sheet SDS201/AD

Date of issue September 2006



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Product name:	Sodium azide R:22-32	CAS No. 26628-22-8 Harmful if swallowed. Contact with acids liberates very toxic gas.
	S: (1/2)-28-45	(Keep locked up and out of the reach of children). After contact with skin, wash immediately with plenty of water. In case of accident or if you feel unwell, seek medical advice immediately (show label where possible).
Composition:	Aqueous sodium azide solution (0.1% - 0.99%)	
Hazards identification:	Harmful if swallowed, inhaled, or absorbed through skin. May cause eye and skin irritation.	
First aid measures:	In case of contact, immediately flush eyes or skin with copious amounts of water. If inhaled remove to fresh air. In severe cases seek medical attention.	
Fire fighting measures:	Dry chemical powder. Do not use water.	
Accidental release:	Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Mop up spill area, place waste in a bag and hold for waste disposal. Wash spill site area after material pick-up is complete.	

Handling and storage:	Wear suitable protective clothing including laboratory overalls, safety glasses and gloves. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling.
Personal protection:	See above instructions for handling and storage.
Physical and chemical properties:	Formula weight: 65.01
Density:	1.850
Stability and reactivity:	Avoid contact with metals and acid chlorides. This yields a very toxic gas.
Toxicological information:	LD ₅₀ : 27mg/kg oral, rat LD ₅₀ : 20mg/kg skin, rabbit
Ecological information:	Not applicable.
Disposal considerations:	Up to 5 vials worth of material may be disposed of directly down the sink with water. If 6 or more vials are to be disposed of they should pass through a chemical waste route.
Transport information:	No special considerations applicable.
Regulatory information:	The information contained in this safety data sheet is based on published sources and is believed to be correct. It should be used as a guide only. It is the responsibility of the user of this product to carry out an assessment of workplace risks, as may be required under national legislation.

For further information, contact your local office.

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