Prostaglandin D_2 [^{3}H] Biotrak Assay System

code TRK 890

100 tubes

STORAGE

Store the reagents at -15°C to -30°C

EXPIRY

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

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.

Amersham Biosciences

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 -15°C to -30°C.

Standard
Prostaglandin D_2 standard, 3ng, lyophilised.

Tracer
[^{3}H]Prostaglandin D_2 ~37kBq, 1µCi, in methanol:water:acetonitrile (3:2:1).

Antiserum
Prostaglandin D_2 antiserum, lyophilised.

Assay buffer
Phosphate buffer concentrate, which on dilution yields approximately 0.05M phosphate buffer pH7.3, containing 0.1% gelatin and 0.1% sodium azide (see safety data sheet on p24).

Dextran coated charcoal
A ready-to-use suspension containing 1% Norit A™ charcoal and 0.5% dextran T70 in 0.01M phosphate buffer pH7.3, containing 0.1% sodium azide (see safety data sheet on p.24).

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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 material 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 regulations or codes of practice which relate to such matters.

Warning: contains sodium azide. See safety data sheet on p.24.

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 or eyes. In the case of contact with skin or eyes wash immediately with water (see safety data sheet for specific advice).

DESCRIPTION

The Biotrak™ prostaglandin D_2 [^{3}H]assay system from Amersham Biosciences has been specifically designed for research purposes.

The system combines the use of a tritiated prostaglandin D_2 tracer with an antiserum which is specific for prostaglandin D_2. This provides a rapid, simple and sensitive method for the determination of prostaglandin D_2 *in vitro* over the range 3 to 200pg/tube. Each pack contains sufficient material for the construction of one standard curve and the assay of 40 unknowns in duplicate.

The assay is based upon competition between unlabelled prostaglandin D_2 and a fixed quantity of tritium labelled prostaglandin D_2 for binding to a limited quantity of an antibody which has high specificity and affinity for prostaglandin D_2. The amount of radioactive ligand bound by the antibody will be inversely proportional to the concentration of added non-radioactive ligand. Measurement of the protein-bound radioactivity enables the amount of unlabelled prostaglandin D_2 in the sample to be determined.

Separation of protein-bound prostaglandin D_2 from the unbound ligand is achieved by adsorption of the free prostaglandin D_2 on to dextran-coated charcoal, followed by centrifugation. Measurement of the radioactivity in the supernatant quantitates the amount of radioactive ligand bound by the antibody. The concentration of unlabelled prostaglandin D_2 in the sample is then calculated from a standard curve. The principle of the assay is illustrated in figure 1.

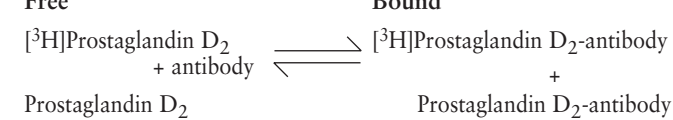

Figure 1.

CRITICAL PARAMETERS

The following points are critical.

- Only Amprep minicolumns should be used with this protocol.
- Stir the charcoal suspension on ice before and during pipetting.
- Standards should be prepared within one hour of performing the assay.

ADDITIONAL EQUIPMENT AND REAGENTS REQUIRED

The following materials and equipment are required:

Refrigerated centrifuge capable of 2000xg
β-Scintillation counter, scintillant (for example Optiphase 'MP'™), scintillant dispenser and counting vials.
Refrigerator
Ice bath
Vortex mixer
Magnetic stirrer and stir bar
Test-tube rack
Disposable polypropylene tubes (12x75mm)
Distilled or deionised water
Pipettes or pipetting equipment with polypropylene tips (100, 200, 300, 500 and 1000µl)
Glass measuring cylinders (10 and 100ml)

SPECIMEN COLLECTION AND SAMPLE PREPARATION

Prostaglandin D_2 is present in a wide variety of biological materials. 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 the literature. The information is provided for guidance only. It remains the investigator's responsibility to validate the chosen sample preparation procedure.

Information describing prostaglandin radioimmunoassays is contained in three relevant articles[25-27]. It is well established that non-esterified fatty acids can interfere with prostaglandin assays[28-30]. Several methods are available for purifying prostaglandins[31-33]. In addition, Dray has evaluated blood collecting procedures[33]. A number of investigators have reported techniques for purifying PGD_2[34-36].

Solid phase extraction procedures such as those described by Powell[32] have become the method of choice for many researchers, giving high recovery and clean samples. An extraction procedure using Amprep™ minicolumns is described below. This protocol has been developed using tracer recovery experiments. The properties of other minicolumns are different so this protocol should only be used with Amprep minicolumns.

Amprep extraction
1) Column type
Amprep C2 100mg code RPN 1903

2) Sorbent conditioning
Rinse the column with 2ml methanol
Rinse the column with 2ml water

3) Sample treatment
Plasma - Acidify 1ml plasma to pH3, apply to the column

4) Interference solution
Wash column with 5ml water
Wash column with 5ml 10% ethanol
Wash column with 5ml hexane (or petroleum ether 30-40°C)

5) Elution
Elute prostaglandin D_2 with 5ml methyl formate

Notes
1) The methyl formate should be dried under nitrogen or vacuum and the extract redissolved in assay buffer before estimation.
2) The typical recovery [^{3}H]prostaglandin D_2 is 90%.
3) For further details of the Amprep range of products see your Amersham Biosciences representative.

ASSAY PROTOCOL

Reagent preparation

Storage

Either distilled or deionised water should be used for reagent preparation. All reagents should be diluted immediately prior to performing the assay.

Excess diluted tracer and reconstituted standards should be frozen after use and stored at -15°C to -30°C. Repeated freezing and thawing should be avoided. Only the most concentrated standard (2ng/ml) should be stored. Reconstituted antiserum, diluted buffer and charcoal suspension should be stored at 2-8°C. These reagents are stable for four weeks under these conditions.

Assay buffer
Allow the vial contents to thaw. Dilute with 60ml of distilled water and mix thoroughly.

Tracer
Dilute the tracer with 10ml of diluted assay buffer and mix thoroughly.

Antiserum
Reconstitute the lyophilised antiserum with 10ml of distilled water. Use a magnetic stirrer to mix the solution for 15 minutes. Ensure that the vial contents are fully dissolved.

Standards
Reconstitute the standard with 1.5ml of distilled water and mix thoroughly. This standard (2ng/ml) serves as the stock solution from which a series of assay standards are prepared by serial dilution.

Charcoal
This will be used on the second day of assay. Thaw the suspension and equilibrate to 2-8°C before use. It may be thawed overnight in a refrigerator.

Preparation of working standards

1) Label 6 polypropylene tubes 3.1pg, 6.2pg, 12.5pg, 25pg, 50pg and 100pg. Pipette 500µl of diluted assay buffer into each.

2) Pipette 500µl of the stock standard into the tube marked 100pg and mix thoroughly.

3) Transfer 500µl from tube 100pg to tube 50pg and mix thoroughly.

4) Repeat this doubling dilution successively with the remaining tubes.

5) 100µl aliquots of each serial dilution will give rise to standard levels of prostaglandin D_2 ranging from 3.1-100pg. The stock solution serves as the top standard of 200pg.

Note: Standards should be prepared within one hour of performing the radioimmunoassay, so as to minimise any effect of prostaglandin D_2 adsorption to the walls of the test tube.

Assay procedure

Day 1
1) Label polypropylene tubes (12x75mm) in duplicate for total counts (TC), non-specific binding tubes (NSB), zero standard tubes (B_0), standards and samples.
2) Starting with the most dilute, pipette 100µl of each standard into the appropriately labelled tubes. Use a new pipette tip for each standard.
3) Pipette 100µl of sample into the appropriately labelled tubes. Use a new pipette tip for each sample.
4) Pipette 100µl of tracer into all tubes.

5) Pipette 100µl of antiserum into all tubes except the non-specific binding tubes.
6) Pipette 100µl of diluted assay buffer into the standard and sample tubes, 200µl into the total counts and zero standard tubes and 300µl into the non-specific binding tubes.
7) Vortex mix all tubes and incubate overnight at 2-8ºC.

Day 2
8) Place all tubes into an ice-water bath and allow to equilibrate for 10 minutes.
9) Place the bottle containing the charcoal into an ice-bath. Gently mix for 10 minutes using a magnetic stirrer.
10) Pipette 500µl of diluted assay buffer into the total count tube.
11) While continuously stirring the charcoal suspension, pipette 500µl of the suspension into all tubes except the total counts.
12) Vortex mix each tube immediately after addition of the charcoal to the last tube.
13) Leave all tubes to stand in the ice-water bath for 10 minutes.
14) Centrifuge all tubes at 2000xg for 10 minutes at 4ºC.
15) After centrifugation, replace all tubes in the ice-water bath and immediately decant the supernatant into scintillation vials. Care should be taken to avoid disturbing the charcoal pellet.
16) Add 10ml of scintillant to each vial, cap, mix and measure the radioactivity for 4 minutes in a β-scintillation counter.

DATA PROCESSING

Calculation of standard curve data

The calculation is illustrated using representative data shown in table 1.

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:

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

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

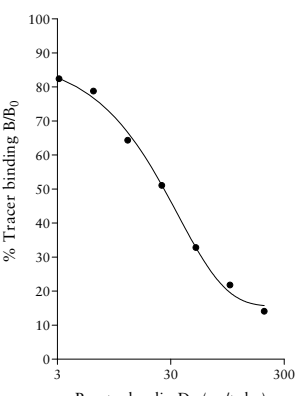

Figure 2.
Prostaglandin D_2 standard curve

Table 1. Standard curve calculation using representative data

Tube	cpm	%B/B_0
Total counts	6777	-
NSB	181	-
Zero standard(B_0)	2444	-
3.10 pg/tube	2046	82.42
6.25 pg/tube	1964	78.81
12.50 pg/tube	1637	64.36
25.00 pg/tube	1337	51.10
50.00 pg/tube	924	32.81
100.00 pg/tube	675	21.81
200.00 pg/tube	500	14.1

ADDITIONAL INFORMATION

Specificity

The specificity data for the prostaglandin D_2 antiserum are shown below:

Table 2. Specificity data

Compound	%Cross-reactivity (50% B/B_0 displacement)
Prostaglandin D_2	100
Prostaglandin J_2	0.4451
Prostaglandin F_{2a}	0.8855
Prostaglandin E_2	<0.03
Prostaglandin F_{1a}	1.5788
Prostaglandin E_1	<0.03
Prostaglandin A_2	<0.03
Thromboxane B_2	0.04329
13,14-Dihydro-15-keto-PGF_{2a}	0.0602
6,15-Diketo-13,14-dihydro-PGF1α	<0.03
6-Keto-PGE1	<0.03
6-Keto-$PGF_{1\alpha}$	0.00116
Arachidonic acid	<0.03
Δ^{12}-PGJ_2	0.8633
15-Deoxy-Δ^{12}-Δ^{14}-PGJ_2	<0.03

Other assay data

Typical assay parameters are listed below:

Specific binding of tracer	33%
Non-specific binding	3%
50% B/B_0 displacement	24pg/tube

Troubleshooting guide

Problem	Check
Poor replication	• Reduce the pipetting speed. Use 'contain' pipetting with tip touch at source and destination. • Ensure that a homogeneous suspension of charcoal is maintained. • If replication is still poor, calibrate equipment. If no improvement is gained, contact supplier of equipment.

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. Prostaglandin D_2 originates from both enzymatic[1,2] and non-enzymatic transformation of prostaglandin endoperoxides[3]. Biosynthesis of PGD_2 occurs in many tissues including platelets[4], bone marrow[5] and mast cells[6].

The biological actions of prostaglandin D_2 include contraction of pulmonary[7] and coronary[8] vascular smooth muscle and inhibition of platelet aggregation[9]. It also increases the chemotactic activity of eosinophil granulocytes[10]. PGD_2 is particularly abundant in brain tissue[11], where it may function as a neuromodulator[12]. Prostaglandin D_2 also has synergistic interactions with other inflammatory mediators[13].

It has been suggested that prostaglandin D_2 is involved in the pathogenesis of bronchoconstriction in allergic asthma[14]. Prostaglandin D_2 is released in substantial quantities after 1gE-dependent challenge of human lung fragments[15]. It is the most abundant cyclo-oxygenase product generated and released after

immunologic[16] and ionophore-induced[17] activation of human pulmonary mast cells. Aerosol administration of prostaglandin D_2 induces bronchoconstriction in animals[18] and humans[14].

The abnormally high urine levels of prostaglandin D_2 metabolites in systemic mastocytosis suggest that prostaglandin D_2 is produced by human mast cells *in vivo*[19].

In addition to their well established role as mediators of inflammation, prostaglandins have more recently been reported to affect cell proliferation, carcinogensis and tumour growth[20]. PGD_2 and its metabolites have been shown to inhibit the growth of a number of tumour cell lines[21,22]. Indeed, PGD_2 appears to be the most potent prostaglandin in this respect[23]. These effects have been observed at levels which do not appear to effect non-malignant cells[24].

The availability of the Biotrak prostaglandin D_2 radioimmunoassay system will facilitate the evaluation of the physiological role of this important arachidonic acid metabolite.

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Related products

Eicosanoids

Thromboxane B_2, [^{3}H]	RIA	TRK 780
Thromboxane B_2, [^{3}H]	SPA	TRK 951
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 B_4, [^{3}H]	RIA	TRK 980
Leukotriene B_4, [^{3}H]	SPA	TRK 954
Leukotriene C_4 specific (Mab), [^{3}H]	RIA	TRK 905
Leukotriene $C_4/D_4/E_4$,	EIA	RPN 224
Leukotriene $C_4/D_4/E_4$, [^{3}H]	RIA	TRK 910
Platelet activating factor (PAF), [^{3}H]	SPA	TRK 990
Prostaglandin F_2, [^{3}H]	RIA	TRK 900
Bicyclic prostaglandin E_2, [^{3}H]	RIA	TRK 800
Prostaglandin E_2	EIA	RPN 222
Prostaglandin E_2, [^{125}I]	RIA/AM	RPA 530
Prostaglandin E_2, [^{125}I]	SPA	RPA 539
6-Keto-prostaglandin $F_{1\alpha}$	EIA	RPN 221
6-Keto-prostaglandin $F_{1\alpha}$ [^{125}I]	RIA/AM	RPA 515
6-Keto-prostaglandin $F_{1\alpha}$ [^{3}H]	SPA	TRK 952
6-Keto-prostaglandin $F_{1\alpha}$ [^{3}H]	RIA	TRK 790

Scintillation proximity assay reagents

Anti-rabbit	RPN 140
Anti-mouse	RPN 141
Anti-sheep	RPN 142
Protein A	RPN 143
Development pack for rabbit antibodies	RPN 144

Amersham Biosciences's range also includes leukotreines, prostaglandins and thromboxanes, labelled archidonic acids and other fatty acids, specifically 2-labelled arachidonyl phospholipids and labelled steroidal and non-steroidal anti-inflammatory drugs.

Amprex C2 100mg	pack of 100	RPN 1903
Amprep column adapter	pack of 15	RPN 1927
Amprep reservoir	pack of 100	RPN 1929
Amprep Manifold-10		RPN 1930
Amprep Super Separator-24		RPN 1940

For further details of Amersham Biosciences's scintillation proximity assay reagents and systems for homogeneous radioimmiunoassays see your Amersham Biosciences representative.

Safety data sheet

SDS201/AA Date of issue Jul 1998

Amersham Biosciences UK Limited Amersham Place Little Chalfont
Buckinghamshire England HP7 9NA Telephone: +44 (0)870 606 1921

Product name: Sodium azide CAS No:26628-22-8

R: 22-32 Harmful if swallowed. Contact with acids liberates very toxic gas.

S: (1/2)-28-45 (Keep locked up and out of 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. See page 26.

Safety data sheet contacts

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