product code:

RPN 222

Biotrak™ Prostaglandin E_2 direct assay kit

NEW IMPROVED VERSION

Benefits of New Version

- **Improved technology:** the new kit contains novel lysis reagents which are proprietary to Amersham Biosciences (lysis reagent 1 and lysis reagent 2). The lysis reagents eliminate time consuming extraction procedures.
- **Flexibility - choice of protocols:** the new kit is better validated and has a wider choice of protocols. The table below summarizes the protocols now available.

	Description	Sample types	Features
Protocol 1	Standard EIA procedure (range 2.5-320pg/well)	urine, cell culture supernate, tissues	
Protocol 2	High sensitivity EIA method (range 1-32pg/well)	tissue samples	
NEW Protocol 3	Direct intracellular PGE_2 method (range 2.5-320pg/well)	cell cultures	lysis reagents avoid time consuming extraction methods
NEW Protocol 4	Total cellular PGE_2 method (intracellular and cell supernate) (range 2.5-320pg/well)	cell cultures	lysis reagents avoid time consuming extraction methods
NEW Protocol 5	Plasma PGE_2 assay (range 2.5-320pg/well)	plasma	lysis reagents avoid time consuming extraction methods

Assay specifications

Dynamic range: 2.5-320pg/well or 1-32pg/well. See protocol choice above

Sensitivity: 16pg/well

Sample types: plasma (EDTA, citrate), urine, tissue homogenates, cell culture and cell culture supernatants

Sample volume: 50µl

Incubation time: 1.5-4.5 hours (depending on protocol)

Protocol summary

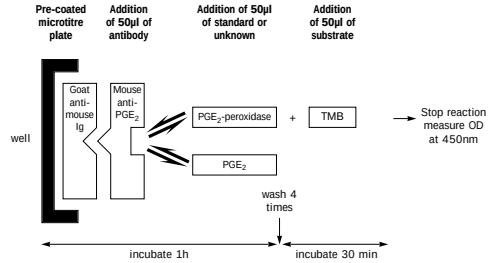

Fig 1 EIA principle.

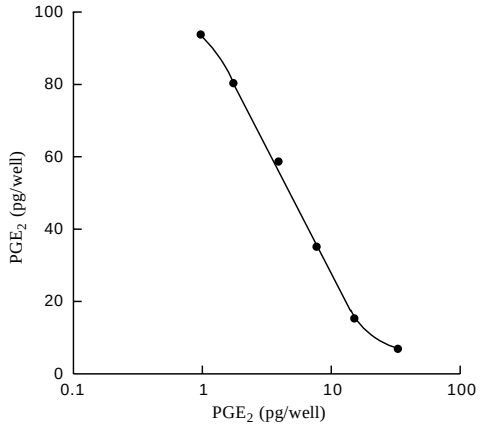

Fig 2 Typical standard curve for 4 hour incubation.

Kit components

- **Pre-coated 96 well microtitre in 12x8 well strip format**
- **PGE_2 standard**
- **Anti-PGE_2 antibody**
- **HRP-conjugated PGE_2**
- **Wash buffer concentrate**
- **Pre-mixed TMB substrate**
- **Lysis reagent 1**
- **Lysis reagent 2**
- **Protocol booklet**

Lysis reagents

Lysis reagent 1 is a detergent which breaks open cell membranes releasing intracellular contents enabling them to be assayed directly. In plasma it disrupts molecular interactions, therefore freeing molecules from association with plasma proteins.

This detergent would also disrupt antibody binding and interfere with the assay. Lysis reagent 2 is key because it sequesters the detergent in Lysis 1, so that results are not affected.

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Key improvements

In plasma studies

In the majority of ELISAs PGE-2 must first be purified away from plasma proteins using time consuming solid phase or solvent extraction. The lysis reagents in RPN 222 eliminate the need for the traditional extraction process but give a recovery of 100% (Figure 3).

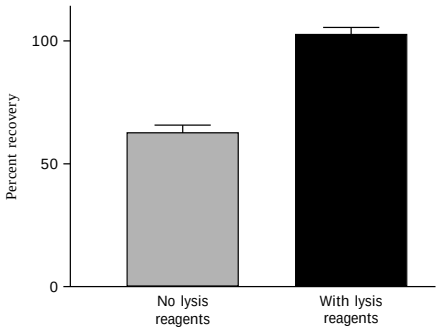

Fig 3 Recovery of PGE-2 from normal plasma. Normal human plasma was spiked with known concentrations of PGE_2 and assayed with and without lysis reagents. Mean recovery of PGE_2 was approximately 55% in the absence of lysis reagents. Recovery of added PGE_2 rose to 100% with the addition of lysis reagents.

Intracellular assay - looking for COX-2 inhibitors in cell cultures

Since PGE_2 is secreted into the culture medium, cell culture supernates are traditionally assayed for PGE_2. Intracellular levels of PGE_2 give a more sensitive and accurate reflection of cellular response than traditional cell supernatant measurements aiding identification of new anti-inflammatory drugs and avoiding false negatives.

To measure the intracellular PGE_2 levels lysis reagent 1 is added to cells in culture after decanting the supernatant. After 5 minutes the PGE_2 is ready to be assayed directly compared to traditional sample extraction techniques which take 12-24 hours to process ~20 samples (Figures 4 and 5).

Total cellular assay (intracellular plus culture supernatant) - cell cultures

Enables the complete picture of cellular response to be obtained as any PGE_2 produced from arachidonic acid will either be in the supernatant or present intracellularly.

To measure the total cellular PGE-2 levels lysis reagents 1 is added to cells in culture without decanting the supernatant. After 5 minutes the PGE_2 is ready to be assayed directly.

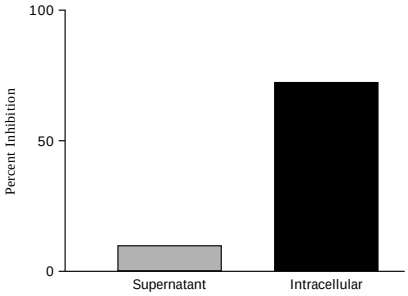

Fig 4 Effect of aspirin on intracellular and PGE_2 generation from mouse 3T3 cells. Mouse 3T3 cells were treated overnight with 4uM aspirin (a COX inhibitor) and then stimulated. Aspirin only reduced the PGE_2 levels in the cell supernatant by 11%, whereas intracellular levels were inhibited 74%.

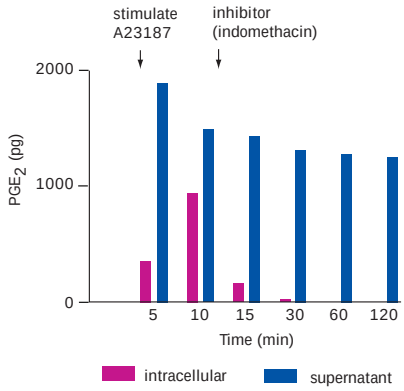

Fig 5 Effect of indomethacin on intracellular and supernatant PGE_2 generation. Mouse 3T3 cells were stimulated before addition of indomethacin (another COX inhibitor). Intracellular PGE_2 decreased significantly on addition of the inhibitor, the supernatant levels only decreased marginally.

Biotrak Guarantee

- **Resulting from 30 years experience in immunoassay development**
- **High Quality assay products you can depend upon**
- **Technical support team on line for practical advice**

The full range of Biotrak products includes assays for: cytokines (human, mouse and rat), MMPs and their inhibitors, 2nd messengers, eicosanoids, cell proliferation, cardiovascular analytes, cell adhesion and steroids

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