Technical tip

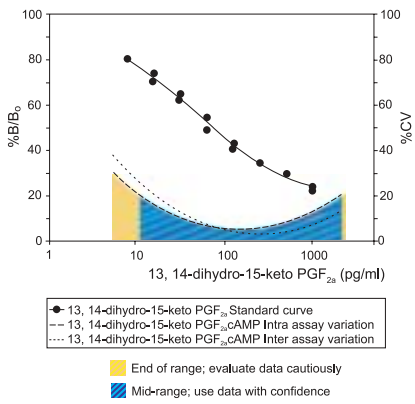
Prostaglandins

Prostaglandin D2 (PGD2) is biosynthesized in the brain by a glutathione-independent lipocalin-type PGD2 synthase (L-PGDS), and one of the primary COX products of arachidonic acid and one of the most widely investigated prostaglandins. It accumulates in the cerebrospinal fluid (CSF), where it induces physiologic sleep in rats and humans. PGD2 is also synthesized by mast cells and leukocytes by a cellular, myeloid-type, glutathione-dependent PGD synthase (hematopoietic PGD Synthase; H-PGDS). In this setting, PGD2 and acts as a pro-inflammatory mediator in allergic reactions following release in large amounts from allergen-stimulated mast cells. Hence, PGE2 is the key indicator of non-steroidal anti-inflammatory drugs efficacy via inhibition of COX-1 and COX-2.

Prostaglandin E2 (PGE2) is one of the primary prostaglandins formed from the coupled metabolism of arachidonic acid by the cyclooxygenases (COX-1 and COX-2) and PGE synthases (microsomal and/or cytosolic).

Prostacyclin (Prostaglandin I2; PGI2) is formed from arachidonic acid primarily by the vascular endothelium and renal cortex. It is a potent vasodilator and inhibitor of platelet aggregation. PGI2 is non-enzymatically hydrated to 6-keto PGF1a ($t_{1/2}$ = 2-3 minutes), and then quickly converted to the major metabolite, 2,3-dinor-6-keto PGF1á ($t_{1/2}$ = 30 minutes). Although 6-keto PGF1á is commonly measured in plasma and urine as an estimate of prostacyclin synthesis, it should be noted that there may be more than one source of PGI2 in these samples. Therefore, it is important to take these factors into account when analyzing data.

Prostaglandin F2α (PGF2a) is one of the five primary PGs derived enzymatically directly from the endoperoxide PGH2. Like all of the primary PGs, PGF2α has a very short half-life in the general circulation. The plasma concentration of PGF2α in humans is <10 pg/ml, and probably no more than 1-2 pg/ml.



All these kits are well documented for sensitivity and specificity - Please inquire.
See also Prostaglandin biochemicals page E87.

Prostaglandin & isoprostane assays

Prostaglandin Screening EIA Kit

All following kits are AchE based EIA, excepted FPIA kits.
All the kits are well documented, especially for sensitivity and specificity. An example of crossreactivities and standard curve is given below. Please inquire for full information.
Kits noted as **"solid plate"** are an economic format: they provide individual reagents as well, but microplates are no already coated.
Kits noted with **"SPE"** : Samples should be purified for some applications (i.e. for certains samples, lowest concentrations, mixture of crossreactive compounds) prior to EIA analysis. Please inquire.
Kits noted with **FPIA/HTS** : Immuno Assay kits are based on Fluorescence Polarisation detection method, and dedicated to numerous samples processing. See complete description after the table of kits.

Description	Qty: 96 wells	Qty: 5x96 wells
Prostaglandin Screening EIA Kit	**Q90723**	**Q90724**
50.80% B/B0: 200-40 pg/ml (crossreacts with all PGs)		
Prostaglandin Screening EIA Kit (solid plate)	**AM1570**	**AM1571**
Prostaglandin D2-MOX EIA Kit	**666703**	**666704**
50-80% B/B0 : 15-3.1 pg/ml		
Prostaglandin D2-MOX EIA Kit (solid plate)	**S00691**	**S00692**
Prostaglandin D2 FPIA Kit – Green See page E207	**GK1640**	**GK1641**
Sens. 470 pg/ml ; Fluorescence Polarization Immunoassay; HTS	(384wells)	(5x384wells)
Prostaglandin D2-MOX Express EIA Kit	**AM1542**	
50-80% B/B0 : 75.2-16 pg/ml; 1 Hr		
Prostaglandin E Metabolite EIA Kit	**833614**	**833613**
50-80% B/B0 : 6-2 pg/ml (converts all major PGE2 metabolites into a single stable derivative) .		
Prostaglandin E Metabolite EIA Kit (solid plate)	**S00720**	**S00721**
Prostaglandin E2 EIA Kit – Monoclonal	**974156**	**974157**
50-80% B/B0 : 51-15 pg/ml		
Prostaglandin E2 EIA Kit – Monoclonal (solid plate)	**S00700**	**S00701**
Prostaglandin E2 Express EIA Kit	**AM1522**	**AM1523**
50-80% B/B0 : 125-36 pg/ml; 1Hr procedure		
Prostaglandin E2 Express EIA Kit (solid plate)	**AM1530**	**AM1531**
Prostaglandin E2 FPIA Kit – Green See page E208	**AM1562**	**AM1563**
Sensib.: 150pg/ml; Fluorescence Polarization Immunoassay; HTS	(384wells)	(5x384wells)
Prostaglandin E2 FPIA Kit – Red See page E208	**GK0350**	**GK0351**
Sensib.: 100pg/ml; Fluorescence Polarization Immunoassay; HTS	(384wells)	(5x384wells)
6-keto Prostaglandin F1 α EIA Kit	**825027**	**825028**
50-80% B/B0 : 43-11 pg/ml		
Prostaglandin F2 α EIA Kit	**866277**	**86626**
50-80% B/B0 : 52-9 pg/ml		
Prostaglandin F2 α EIA Kit (solid plate)	**S00740**	**S00741**
11β-Prostaglandin F2 α EIA Kit	**Q90762**	**Q90763**
50-80% B/B0 : 32-5.5 pg/ml		
11b-Prostaglandin F2 α EIA Kit (solid plate)	**S00760**	**S00761**
8-Isoprostane EIA Kit	**969813**	**969814**
50-80% B/B0: 30-5 pg/ml working range of 7.8-1 000 pg/ml); SPE		
8-Isoprostane EIA Kit (solid plate)	**S00750**	**S00751**
STAT-8-Isoprostane EIA Kit (fast assay)	**AM1553**	**AM1554**
50-80% B/B0: 180-45 pg/ml		
Latanoprost EIA Kit	**S00770**	**S00771**
50-80% B/B0 58-15 pg/ml; SPE		
Latanoprost EIA Kit (solid plate)	**S00780**	**S00781**
(+)-Fluprostenol EIA Kit	**AM1582**	**AM1583**
50-80% B/B0: 110-16 pg/ml		
13,14-dihydro-15-keto Prostaglandin F2 α EIA Kit (solid plate)	**J84683**	**J84684**
50-80% B/B0 : 75-8.2 pg/ml (10 pg/ml)		
Bimatoprost EIA Kit	**J84893**	**J84894**
50-80% B/B0 : 15.5-2.6 pg/ml; SPE		

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Related product :

Description	Cat.#	Qty
8-Isoprostane Affinity PurificationKit	**Q90750**	1u
	Q90751	5u

contains all reagents necessary for simple one-step purification of 8-isoprostane from most biological samples.

Prostaglandin D2 FPIA Kit

Our PGD2 Fluorescence Polarization Immunoassay (FPIA) is especially designed for High Throughput Screening (HTS) applications for direct, rapid measurement of PGD2. The assay is particularly suited for samples from cell culture and purified enzyme preparations. The PGD2-FPIA is robust (Z' = 0.74), exhibits D120 mP over a range of 244 pg/ml to 1 000 ng/ml PGD2, and has a detection limit of 470 pg/ml.

Specificity

Prostaglandin D2-MOX	100%
Prostaglandin D2	0.2%
Prostaglandin E2-MOX	<0.01%
6-keto Prostaglandin F1a-MOX	<0.01%
Prostaglandin F2a?	<0.01%
Tetranor-PGEM	<0.01%
Tetranor-PGFM	<0.01%
Thromboxane B2-MOX	<0.01%

Description	Cat.#	Qty
Prostaglandin D2 FPIA Kit – Green	GK1640	384 wells
	GK1641	5 x 384 wells

Sens. 470 pg/ml ; Fluorescence Polarization Immunoassay; HTS

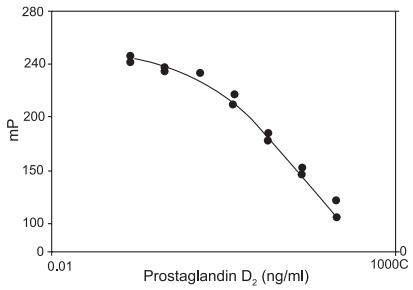
Technical tip

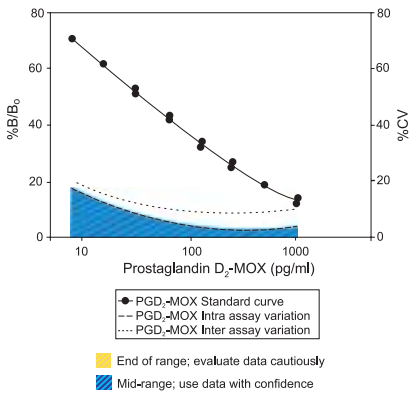
Isoprostanes
The **isoprostanes** are a family of eicosanoids of non-enzymatic origin produced by the random oxidation of tissue phospholipids by oxygen radicals. Isoprostanes appear as artifacts in tissue and plasma samples which have undergone oxidative degradation during prolonged or improper storage. They also appear in the plasma and urine under normal conditions and are elevated by oxidative stress. At least one of the isoprostanes, 8-isoprostane (8-epi PGF2á), has been shown to have biological activity. Plasma from healthy volunteers contains modest amounts of 8-isoprostane (40-100 pg/ml) that increase with the age of the test subject. Normal human urinary levels range from 10-50 ng/mmol creatinine, which is an order of magnitude higher than many enzymatically derived eicosanoids.

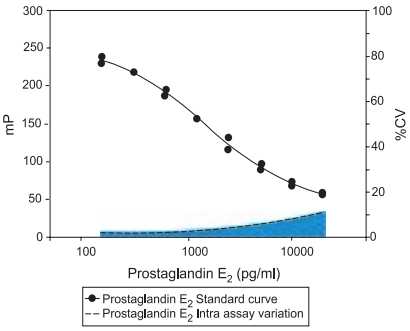
Latanoprost is 13,14-dihydro-17-phenyl-18,19,20-trinor prostaglandin F2a isopropyl ester (13,14-dihydro-17-phenyl-18,19,20-trinor PGF2a isopropyl ester), an F-series prostaglandin analog which has been approved for use as an ocular hypotensive drug. Prostaglandin esters and other similar derivatives act as prodrugs which are converted to the active free acid form by an esterase/amidase activity in ocular tissues.

(+)-**Fluprostenol** is a metabolically stable analog of PGF2a with potent FP receptor agonist activity. (+)-Fluprostenol isopropyl ester is converted by esterase activity in the cornea to yield the corresponding free acid.

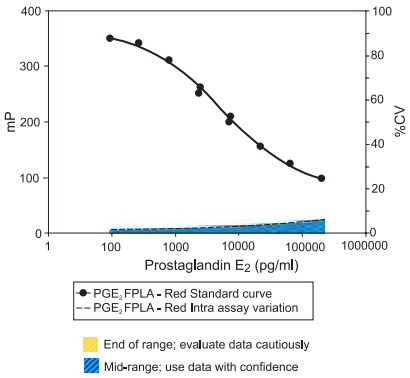
Bimatoprost (free acid) (17-phenyl trinor PGF2a) is a metabolically stable analog of PGF2a and is a potent agonist for the FP receptor.







Standard curve for kit #AM1562



Standard curve for kit #GK0350

Prostaglandin E2 FPIA Kits

PGE2 Fluorescence Polarization Immunoassay (FPIA) is a break-through method for rapid, high-throughput screening of PGE2 samples. This FPIA is a homogenous, single-step assay which is easily adaptable to robotic assay systems. Simply mix the sample or standard with the assay cocktail, incubate at least 60 minutes, and read the plate whenever you are ready. Each kit contains FPIA reagent, buffer concentrate (10X), standard, 384 well plate, foil plate cover, and complete instructions.

The kit #GK035 uses the red-shifted dye rhodamine as the label, rather than fluorescein, which is used in the original PGE2 FPIA Kit - Green # AM156é. The red-shifted dyes offer the advantage of limited interference from most compounds in a drug screening library, as well as reduced interference from sample matrices. Samples from cell culture and recombinant protein expression systems may require modest dilutions (1:5-1:20) and no purification prior to being used. Samples containing no phenol red or serum can be assayed without any dilution. Using this assay it is possible to rapidly analyze samples and increase the rate of lead compound identification without time-consuming incubation, washing, and development steps and without complex interference problems.

Specificity

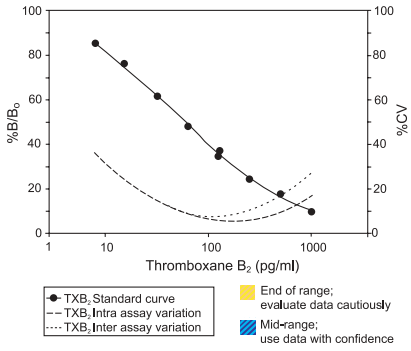
Prostaglandin E1	100%
Prostaglandin E2 Ethanolamide	100%
Prostaglandin E3	85.0%
Sulprostone	9.0%
6-keto Prostaglandin F1a	2.9%
8-iso Prostaglandin F2a	0.09%
Prostaglandin D2	<0.01%
8-iso Prostaglandin E2	<0.01%

Description	Cat.#	Qty
Prostaglandin E2 FPIA Kit – Green	**AM1562**	384 wells
	AM1563	5 x 384 wells
Detection limit : 150pg/ml		
Prostaglandin E2 FPIA Kit – Red	**GK0350**	384 wells
	GK0351	5 x 384 wells
Ddetection limit : 100pg/ml		

Thromboxane assays

All following kits are AchE based EIA, and are well documented, especially for sensitivity and specificity. A Please inquire for full information.
Kits noted as **"solid plate"** are an economic format : they provide individual reagents as well, but microplates are no already coated.

Description	Qty : 96 wells	Qty : 5 x 96 wells
Thromboxane B2 EIA Kit 50-80% B/B0 : 57-11 pg/ml	**803855**	**803856**
Thromboxane B2 EIA Kit (solid plate)	**S00790**	**S00791**
Thromboxane B2 Express EIA Kit – Monoclonal	**GK0070**	**GK0071**
Thromboxane B2 Express EIA Kit – Monoclonal (solid plate)	**BG8150**	**BG8151**
11-dehydro Thromboxane B2 EIA Kit 50-80% B/B0 : 57-11 pg/ml	**800715**	**800716**
11-dehydro Thromboxane B2 EIA Kit (solid plate)	**S00810**	**S00811**
2,3-dinor Thromboxane B2 EIA Kit 50-80% B/B0 : 112-23 pg/ml	**317094**	**317095**
2,3-dinor Thromboxane B2 EIA Kit (solid plate)	**S00800**	**S00801**



Calibration curve for kit #803855

Technical tip

Thromboxanes
Thromboxane A2 (TXA2) is produced from arachidonic acid by many cells and causes irreversible platelet aggregation and vascular and bronchial smooth muscle contraction.

Thromboxane B2 TXB2 measurement is better suited towards samples that are not expected to undergo extensive metabolism such as perfusates, lavage samples, tissue/cell culture, etc.

2,3-dinor TXB2 will give a more episodic indication of TXA2 production. Because plasma concentrations of 2,3-dinor TXB2 are low (and artifactual TXB2 concentrations may be high), it may be necessary to purify and concentrate plasma samples. It is also recommended to purify urine samples to remove possible crossreacting metabolites and TXB2 from the sample.

Cox Inhibitor Screening Assay Kits

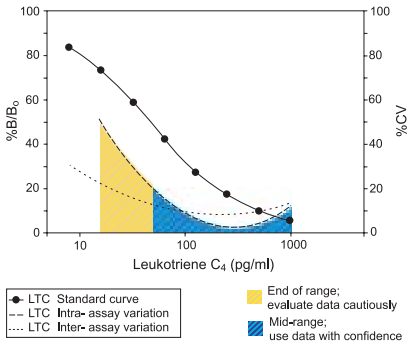
COX Inhibitor Screening Assay

The COX Inhibitor Screening Assay directly measures PGF2α produced in the cyclooxygenase reaction. The prostanoid product is quantified via enzyme immunoassay (EIA) using a broadly specific antibody that binds to all the major prostaglandin compounds. Thus, this COX assay is more accurate and reliable than an assay based on peroxidase inhibition. The COX Inhibitor Screening Assay includes both ovine COX-1 and COX-2 enzymes in order to screen isozyme-specific inhibitors. This assay is an excellent tool which can be used for general inhibitor screening, or to eliminate false positive leads generated by less specific methods.

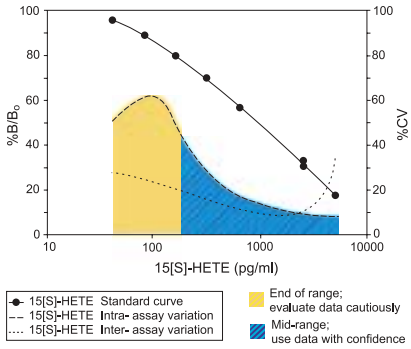
Description	Cat.#	Qty
COX (ovine) Inhibitor Screening Assay	**T92921**	1 kit

Also exist in format with colorimetric (Q91432) and chemiluminescent detection (S03651). Various species available. Please inquire.

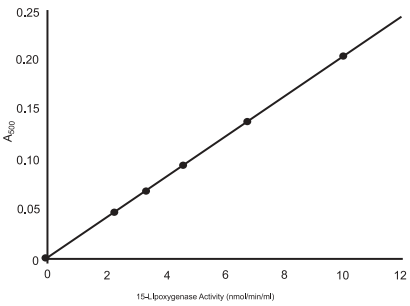
See also COX activity assays page E218.



Leukotriene C4 EIA Kit #869524 calibration curve.



Calibration curve of kit #AM1592



Calibration curve of kit #Q91481

Leukotriene & Lipoxygenase assays

All following kits are AchE based EIA, and are well documented, especially for sensitivity and specificity. Please inquire for full information.
Kits noted as **"solid plate"** are an economic format : they provide individual reagents as well, but microplates are no already coated.

Description	Qty : 96 wells	Qty : 5 x 96 wells
Leukotriene B4 EIA Kit	**852474**	**852475**
50-80% B/B0: 50-13 pg/ml		
Leukotriene B4 EIA Kit (solid plate)	**S00820**	**S00821**
Leukotriene B4 EIA Kit (solid plate)	**S00820**	**S00821**
Leukotriene C4 EIA Kit	**869524**	**869525**
50-80% B/B0 : 45-10 pg/ml		
Leukotriene C4 EIA Kit (solid plate)	**S00830**	**S00831**
Leukotriene C4 EIA Kit (solid plate)	**S00830**	**S00831**
Leukotriene E4 EIA Kit	**869534**	**869535**
50-80% B/B0 : 125-30 pg/ml		
Leukotriene E4 EIA Kit (solid plate)	**S00840**	**S00841**
Cysteinyl-Leukotriene EIA Kit	**Q90772**	**Q90773**
Cysteinyl-Leukotriene EIA Kit (solid plate)	**S00850**	**S00851**
15(S)-HETE EIA Kit	**AM1592**	**AM1593**
50-80% B/B0: 960-170 pg/ml (10-0.1 ng/ml)		
15(S)-HETE EIA Kit (solid plate)	**T91040**	**T91041**
15(S)-HETE EIA Kit (solid plate)	**T91040**	**T91041**
Lipoxygenase Inhibitor Screening Assay Kit	**Q91481**	

Technical tip

Leukotrienes & Lipoxygenases
The **leukotrienes** (LTs) were discovered in 1979 as a group of acute inflammatory mediators derived from arachidonic acid in leukocytes.
Leukotriene B4 (**LTB4**) is synthesized from arachidonic acid by the combined action of 5-lipoxygenase and LTA4 hydrolase. LTB4 has long been recognized as a potent mediator of inflammation. Plasma levels of LTB4 increase from <100 pg/ml to >100 ng/ml following leukocyte stimulation. LTB4 is metabolized in leukocytes and hepatocytes to less active 20-hydroxy and 20-carboxy LTB4 by NADPH-dependent cytochrome P450 enzymes followed by b-oxidation at the w-end to w-carboxy dinor LTB4 and w-carboxy tetranor LTB3. LTB4 is not excreted in the urine.
The conjugation of glutathione to LTA4 results in the formation of LTC4. LTC4 is rapidly metabolized to LTD4 and LTE4. This metabolism is essentially complete within 10 minutes in the human lung. **LTC4** and **LTD4** are potent mediators of asthma and hypersensitivity. They induce bronchoconstriction, increase microvascular permeability, and are vasoconstrictors of coronary arteries. Cys-LTs can accumulate to relatively high concentrations in the effusion fluids associated with inflammation (e.g., ascities fluid, synovial fluid, pleural effusion, pericardial or cerebral aspirates). Cys-LTs are excreted in urine as intact LTE4 (~9-12%) and LTE4 metabolites.
Leukotriene E4 (**LTE4**) is a product of the 5-lipoxygenase (5-LO) pathway in activated mast cells, eosinophils, and monocytes. LTA4, the primary 5-LO metabolite, is converted to LTC4 and sequentially to LTD4 and LTE4 in the host cell, or by transcellular metabolism in erythrocytes, platelets, or neutrophils.

Lipoxygenases catalyze the addition of molecular oxygen to fatty acids containing a cis,cis-1,4-pentadiene system to give an unsaturated fatty acid hydroperoxide. In mammals, lipoxygenases carry out the first step in the arachidonic acid cascade. 5- and 15-LOs lead to the biologically active lipoxins, whereas 5-LO leads to 5,6-epoxy-leukotrienes which are involved in a variety of inflammatory responses, including neutrophil chemotaxis, vascular permeability, and smooth muscle contraction. In contrast, Nassar, et al., suggested that animal 15-LO products act as anti-inflammatory agents. This implies that the 5- and 15-LO pathways may play a role in regulating inflammation. To elucidate the role of each lipoxygenase, it is particularly important to develop inhibitors that are enzyme specific, as allowed by our EIA kit #Q9148.

15(S)-HETE is produced from arachidonic acid by the enzyme 15-lipoxygenase (15-LO). In humans it is formed primarily in the respiratory epithelium, leukocytes, and reticulocytes. 15(S)-HETE has been detected in high concentrations in nasal secretions and may contribute to allergic rhinitis. 15(S)-HETE has anti-inflammatory properties, inhibiting carrageenan-induced arthritis and lowering leukotriene B4 (LTB4) concentrations in the synovial fluid of dogs. It may regulate T-lymphocytes by inhibiting 5- and 12-LOs. It is also a vasoconstrictor, constricting cerebral and coronary arteries of dogs in vitro and cerebral arteries of pigs in vivo. 15(S)-HETE may also play a role in cancer, inhibiting apoptosis by carcinosarcoma cells.

Related products
A vast literature strongly supports pivotal roles for caspase 1 and IL1-beta in inflammation and ischemia. Please review Caspase assays in section "Apoptosis" pages E156-171, and IL1 assays in section ".

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