

# Product Information



## 8-Isoprostane Affinity Column

Catalog No. 10010366 • Lot No. XXXXXXXX

### IMPORTANT

Water used to prepare all EIA reagents and buffers must be deionized and free of trace organic contaminants ("UltraPure"). Use activated carbon filter cartridges or other organic scavengers. Glass distilled water (even if double distilled), HPLC-grade water, and sterile water (for injections) are not adequate for EIA. UltraPure water may also be purchased (Catalog No. 400000).

### Laboratory Procedures

For long term storage, we suggest that the column be stored at 4°C. Be certain that the column is stored in an upright position. This column will be stable for at least two years. The 4 ml column contains 0.5 ml sorbent and the 20 ml column contains 1 ml sorbent. Prior to use, prepare the reagents as described below.

#### 1. 8-Isoprostane Affinity Column

Remove the top stopper and then gently remove the bottom plug. Be certain to remove the top stopper first to avoid air bubbles being drawn into the packing material. Allow the storage solution to pass through the sorbent and then wash the column with 2 ml (4 ml column) or 4 ml (20 ml column), of column buffer. Repeat wash. The column is now ready to use.

#### 2. Eicosanoid Affinity Column Buffer

Prepare a 0.1 M phosphate buffer solution by combining 13.3 g  $K_2HPO_4$ , 3.22 g  $KH_2PO_4$ , 0.5 g  $NaN_3$ , and 29.2 g NaCl. Dilute to a total volume of 1.0 liter with UltraPure water. The pH of this buffer will be 7.4. This buffer may also be purchased as a 5X concentrated buffer (Catalog No. 400220).

#### 3. Eicosanoid Affinity Column Elution Solution

Prepare a solution containing 95% absolute ethanol and 5% UltraPure water. This solution may also be purchased (Catalog No. 400230).

#### 4. EIA Buffer

Prepare a 1.0 M phosphate buffer solution by combining 133 g  $K_2HPO_4$  and 32.15 g  $KH_2PO_4$  and diluting to a total volume of 1.0 liter with UltraPure water. To 100 ml of this buffer, add the following: 100 mg  $NaN_3$ , 23.4 g NaCl, 370 mg tetrasodium EDTA, and 1 g bovine serum albumin (Sigma 7030 or equivalent). Stir at room temperature until completely dissolved and dilute to a total volume of 1.0 liter with UltraPure water; the pH of this solution will be ~7.0. This buffer may also be purchased as a 10X concentrated buffer (Catalog No. 400060).

### Purification Protocol

- All samples must be free of particulates and precipitates to avoid plugging the column. This can be achieved either by filtration or by centrifugation. All samples must be at approximately neutral pH (6.5-7.5). Urine samples should be centrifuged briefly to remove sediment and may be applied directly to the column. Plasma samples should be diluted 1:5 with Column Buffer and applied to the column. Allow the entire sample to pass through the packing material. [NOTE: This affinity column may be used to isolate free 8-isoprostane from samples. If membrane-bound or total 8-isoprostane measurement is desired, please see the 8-Isoprostane EIA Kit booklet (Catalog No. 516351).] The binding capacity of the column is 10 ng of 8-isoprostane per ml of gel sorbent. Your sample should contain an amount of 8-isoprostane equal to 20-50% of the column capacity. For example, 5 ml of a normal urine sample having 600 pg/ml 8-isoprostane would be 30% of the column capacity (for the 20 ml column containing 1 ml of sorbent; a 4 ml column contains 0.5 ml sorbent and can bind 5 ng of 8-isoprostane).
- Wash the 4 ml column with 2 ml Column Buffer, followed by 2 ml UltraPure water. Wash the 20 ml column with 10 ml Column Buffer, followed by 10 ml UltraPure water. Allow all of the water to pass through the packing material. Discard both of these washes.
- Elute the 8-isoprostane from the 4 ml column by adding 2 x 2 ml Elution Solution and allowing it to pass through the packing material. Elute the 8-isoprostane from the 20 ml column by adding 2 x 5 ml Elution Solution and allowing it to pass through the packing material. If the analysis cannot be performed at once store the eluant at -80°C; they will be stable for at least one year.

**WARNING: THIS PRODUCT IS FOR LABORATORY RESEARCH ONLY: NOT FOR ADMINISTRATION TO HUMANS. NOT FOR HUMAN OR VETERINARY DIAGNOSTIC OR THERAPEUTIC USE.**

#### MATERIAL SAFETY DATA

This material should be considered hazardous until information to the contrary becomes available. Do not ingest, swallow, or inhale. Do not get in eyes, on skin, or on clothing. Wash thoroughly after handling. This information contains some, but not all, of the information required for the safe and proper use of this material. Before use, the user must review the complete Material Safety Data Sheet, which has been sent under separate cover to the MSD supervisor at your institution.

#### WARRANTY AND LIMITATION OF REMEDY

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery.

Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence.

This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees. Buyer's exclusive remedy and Cayman's sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman's option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

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- Evaporate the Elution Solution to dryness either by vacuum centrifugation or by evaporation under a stream of dry nitrogen. It is imperative that all of the organic solvent be removed as even trace quantities may adversely affect any immunoassay.
- Immediately dissolve the 8-isoprostane in the buffer or solvent appropriate for your application. If you are assaying the sample with our 8-isoprostane EIA kit (Catalog No. 516351), dissolve the sample in EIA Buffer. The amount of EIA Buffer depends on the original sample volume and the expected concentration of 8-isoprostane in the sample. A dilute sample may be concentrated by dissolving the residue in a smaller volume of EIA Buffer than the original sample volume.
- Regenerate the 4 ml column by washing with 5 ml UltraPure water, and then 5 ml Column Buffer. Regenerate the 20 ml column by washing with 10 ml UltraPure water, and then 10 ml Column Buffer. Store the column at 4°C with 1-2 ml column buffer in the column to keep the packing material from becoming dry. The tip cap must be placed very tightly on the bottom of the column to ensure that the Column Buffer does not leak out and allow the packing material to become dry. The top stopper should be inserted last and removed first to keep air bubbles from entering the packing material. Each column can be used up to four times when purifying 200 µl of plasma on a 4 ml column. Larger volumes of plasma will decrease the number of times the column can be successfully regenerated. For urine samples, each column should be limited to a single use. Recovery of 8-isoprostane from 2 ml of first morning urine was ≥90%. Each regeneration cycle changes (decreases) the sorbent binding capacity, so fresh and regenerated columns are not strictly comparable. We urge you to use identical column lots for each study or data set.

#### Recovery

Urine and plasma recoveries average >90% with a variance of <20%.

#### Sample Data

The data shown in the figure below was generated by purifying 8-isoprostane from urine or plasma using the affinity column. Urine or plasma was spiked with the indicated amounts of 8-isoprostane and purifications were performed for each concentration. Samples were analyzed by EIA.

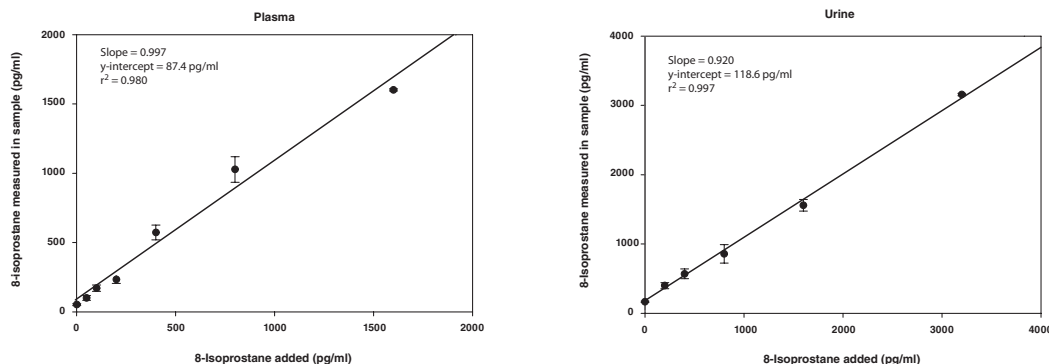


Figure 1. Recovery of 8-Isoprostane in plasma and urine

#### Specificity

8-Isoprostane	100%	8,12- <i>epi</i> iPF <sub>2α</sub> -VI	<0.01%
8- <i>iso</i> Prostaglandin F <sub>3α</sub>	7.6%	Leukotriene E <sub>4</sub>	<0.01%
Prostaglandin F <sub>1α</sub>	2.85%	8- <i>iso</i> Prostaglandin E <sub>2</sub>	<0.01%
Prostaglandin F <sub>2α</sub>	0.88%	13,14-dihydro-15-keto Prostaglandin E <sub>2</sub>	<0.01%
11β-Prostaglandin F <sub>2α</sub>	0.83%	2,3-dinor-6-keto Prostaglandin F <sub>1α</sub>	<0.01%
Prostaglandin E <sub>2</sub>	0.34%	2,3-dinor-8- <i>iso</i> Prostaglandin F <sub>1α</sub>	<0.01%
Prostaglandin E <sub>1</sub>	0.32%	6-keto Prostaglandin F <sub>1α</sub>	<0.01%
8- <i>iso</i> Prostaglandin E <sub>1</sub>	0.14%	13,14-dihydro-15-keto Prostaglandin F <sub>2α</sub>	<0.01%
8,12- <i>epi</i> iPF <sub>2α</sub> -III	0.03%	2,3-dinor Thromboxane B <sub>2</sub>	<0.01%
iPF <sub>2α</sub> -VI	<0.01%	11-dehydro Thromboxane B <sub>2</sub>	<0.01%

#### Related Products

8-Isoprostane Affinity Purification Kit (4 ml) - Cat. No. 10367 • 8-Isoprostane Affinity Purification Kit (20 ml) - Cat. No. 10368 • 8-*iso* Prostaglandin F<sub>2α</sub> - Cat. No. 16350 • UltraPure Water - Cat. No. 400000 • Eicosanoid Affinity Column Buffer (5X) - Cat. No. 400220 • Eicosanoid Affinity Column Elution Solution - Cat. No. 400230 • 8-Isoprostane EIA Kit - Cat. No. 516351 • 8-Isoprostane Affinity Sorbent - Cat. No. 10010365

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# Product Information



## Analytical Data Sheet

### 8-Isoprostane Affinity Sorbent

Catalog No. 10010366 • Lot No. XXXXXXXX

Date of Manufacture: XX/XX/XX

#### Assay Conditions

1. Eicosanoid Affinity Column Buffer (Catalog No. 400220) was used to prepare an 8-isoprostane solution at 10 ng/ml.
2. Affinity columns were prepared following Cayman's standard protocol.
3. One column was used for purification of each concentration of 8-isoprostane. All fractions from the purification procedure were saved and 8-isoprostane levels in the fractions were measured by EIA.
4. To perform the purifications:
  - a. Columns were equilibrated with Column Buffer (Catalog No. 400220).
  - b. Columns were washed with 2 x 2ml of Column Buffer.
  - c. One ml of the appropriate 8-isoprostane containing solution was applied to each column.
  - d. Columns were washed with 2 ml Column Buffer.
  - e. Columns were washed with 2 ml UltraPure water.
  - f. The isoprostane was eluted with 2 ml Elution Solution (Catalog No. 400230).
  - g. The Elution Solution was evaporated under a stream nitrogen and reconstituted in 2 ml EIA buffer (Catalog No. 400060).
  - h. All other were measured by EIA after performing any appropriate dilutions in EIA buffer.

#### Assay Results

Results are reported as % of total 8-Isoprostane

Concentration of isoprostane	
Sample	10 ng/ml
Flow through	0.15%
Wash	0.07%
Wash 2	0.13%
Elution	99.6%

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