

Product Information



8-Isoprostane Affinity Purification Kit (20 ml)

Item No. 10368

Table of Contents

Item No.	Item Name	1 ea Quantity/Size	5 ea Quantity/Size
400220	Eicosanoid Affinity Column Buffer (5X)	1 vial/50 ml	1 vial/250 ml
400230	Eicosanoid Affinity Column Elution Solution	1 vial/50 ml	1 vial/250 ml
400059	8-Isoprostane Affinity Column	1 column/1 ml sorbent in a 20 ml column	5 columns/1 ml sorbent in a 20 ml column

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

Precautions

Please read these instructions carefully before beginning the purification.

For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information

Phone: 888-526-5351 (USA and Canada only) or 734-975-3888

Fax: 734-971-3641

Email: techserv@caymanchem.com

Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability

This kit will perform as specified if stored at 4°C and used before the expiration date indicated on the outside of the box.

Reagent Preparation

1. Eicosanoid Affinity Column Buffer (5X) (Item No. 400220)

Dilute 1:5 with UltraPure water (*i.e.*, dilute 50 ml vials to a total volume of 250 ml and the 250 ml vials to a total volume of 1,250 ml).

2. 8-Isoprostane Affinity Column (Item No. 400059)

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 gel and then wash two times with 4 ml of Column Buffer. The solution will stop flowing once the fluid level reaches the top of the upper frit. The column is now ready to use.

3. Eicosanoid Affinity Column Elution Solution (Item No. 400230)

This solution is ready to use.

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 *via* email to 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.

For further details, please refer to our **Warranty and Limitation of Remedy** located on our website and in our catalog.

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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. The pH of this solution will be 7.4. To 100 ml of this buffer, add the following: 100 mg sodium azide, 23.4 g sodium chloride, 370 mg tetrasodium EDTA, and 1 g bovine serum albumin (Sigma A7030 or equivalent). Stir at room temperature until completely dissolved and dilute to a total volume of 1.0 liter with UltraPure water. This buffer may also be purchased (Item No. 400060).

Purification Protocol

1. All samples must be free of particulates and precipitates. 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 (Item No. 516351).* The binding capacity of the column is 10 ng of 8-isoprostane. 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.
2. Wash the 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.
3. Place a test tube capable of holding at least 10 ml under the column. Elute the 8-isoprostane from the 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 $-80^{\circ}C$; it will be stable for one year.)
4. 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.
5. Immediately dissolve the sample in the buffer or solvent appropriate for your application. If you are assaying the sample with our 8-Isoprostane EIA Kit (Item 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 solvent than the original sample volume.
6. Regenerate the column by washing with 10 ml UltraPure water, and then 10 ml Column Buffer. Store the column at $4^{\circ}C$ with 2-4 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 400 μ l of plasma on a 20 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. Each regeneration cycle decreases the sorbent binding capacity, so fresh and regenerated columns are not strictly comparable. We urge you to use identical columns for each study or data set.

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Recovery

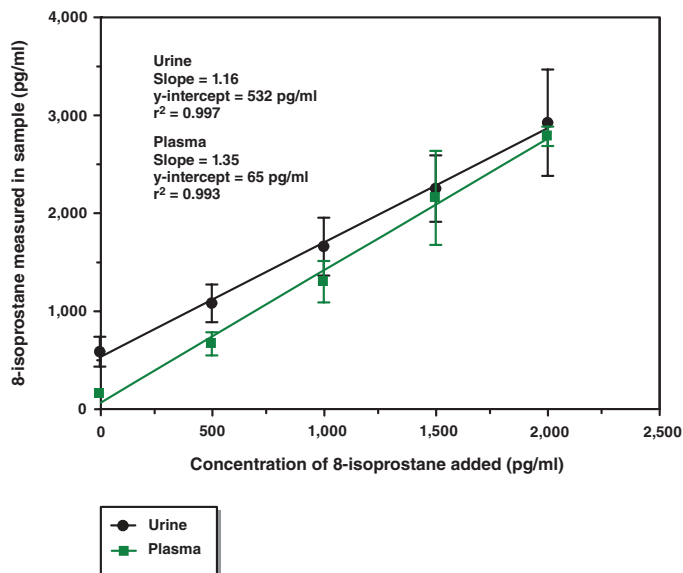


Figure 1. Recovery of 8-isoprostane from urine and plasma following immunoaffinity purification. Urine and plasma samples were spiked with 8-isoprostane, purified using immunoaffinity chromatography, and analyzed using the 8-Isoprostane EIA Kit. The y-intercepts correspond to the amount of 8-isoprostane measured in the unspiked samples.

Specificity

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

Related Products

- 8-Isoprostane Affinity Purification Kit (4 ml) - Item No. 10367
- 8-*iso* Prostaglandin F_{2 α} - Item No. 16350
- UltraPure Water - Item No. 400000
- 8-Isoprostane Affinity Column (4 ml) - Item No. 400058
- 8-Isoprostane Affinity Column (20 ml) - Item No. 400059
- Eicosanoid Affinity Column Buffer (5X) - Item No. 400220
- Eicosanoid Affinity Column Elution Solution - Item No. 400230
- 8-Isoprostane EIA Kit - Item No. 516351
- 8-Isoprostane Affinity Sorbent - Item No. 10010365

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