

8-Isoprostane Affinity Purification Kit (4 ml)

Catalog No. 10009944

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CONTENTS OF THE KIT

Item Number	Item	Quantity/Size	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
416358	8-Isoprostane Affinity Column	1 column/0.5 ml sorbent	5 columns/0.5 ml sorbent

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

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

- Please read these instructions carefully before beginning this assay.
- For research use only. Not for human or diagnostic use.

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.

IF YOU HAVE PROBLEMS

Our technical service staff may be reached by phone (888-526-5351, 734-975-3888), fax (734-971-3641), or E-Mail (techserv@caymanchem.com) Monday through Friday 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

For long term storage, we suggest that this kit be stored at 4°C. Be certain that the column is stored in an upright position. This kit will be stable for at least two years. Prior to use, prepare the reagents as described below.

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).

REAGENT PREPARATION

1. 8-Isoprostane Affinity Column (Catalog No. 416358)

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 the 4 ml column with 2 ml of column buffer. Repeat the wash. The column is now ready to use.

2. Eicosanoid Affinity Column Buffer (5X) (Catalog 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).

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

This solution is ready-to-use.

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 7030 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 (Catalog No. 400060).

PURIFICATION PROTOCOL

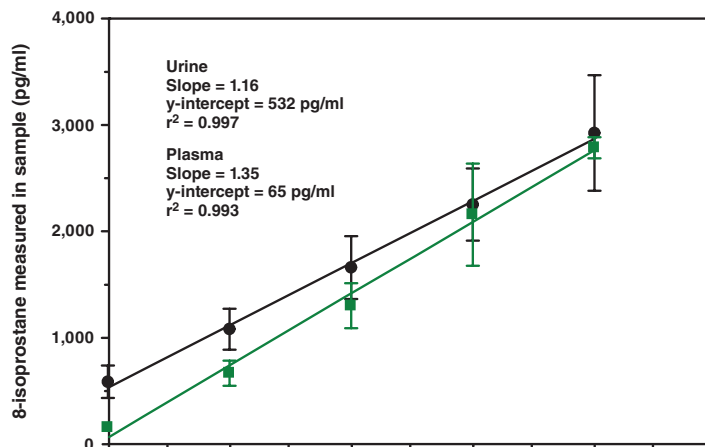
1. All samples must be free of particulates and precipitates to avoid plugging the upper frit. This can be achieved either by filtration or by centrifugation. Urine samples may be applied directly to the column. Plasma samples should be diluted 1:5 with Column Buffer and applied to the column. Allow 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 containing 0.5 ml of sorbent is 5 ng of 8-isoprostane. Your sample should contain an amount of 8-isoprostane equal to 20-50% of the column capacity. For example 3 ml of a normal urine sample having 600 pg/ml 8-isoprostane would be ~36% of the column capacity.
2. Wash the column with 2 ml Column Buffer, followed by 2 ml UltraPure water. Repeat wash. 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 5 ml under the column. Elute the 8-isoprostane from the column by adding 2 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; 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 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.
6. Regenerate the column by washing with 5 ml UltraPure water, and then 5 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 found to be ≥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%. If your application requires greater precision, samples may be spiked with tritium labeled ($[^3\text{H}]$) 8-isoprostane prior to purification. For a protocol on recovery estimation using tritiated $[^3\text{H}]$ -8-isoprostane, please see the 8-Isoprostane EIA Kit booklet (Catalog No. 516351).

SAMPLE DATA

The data shown in Figure 1 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. The average recoveries of 8-isoprostane spiked into human urine and plasma at concentrations from 500-2,000 pg/ml were 118% and 120%, respectively. (The recovery of 8-isoprostane in excess of 100% possibly indicates that the sorbent also binds a small amount of an unknown isoprostane metabolite closely related to 8-isoprostane.)



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%
11β-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-*iso* Prostaglandin F_{2α} - Cat. No. 16350 • [³H]-8-*iso* Prostaglandin F_{2α} - Cat. No. 216350 • UltraPure Water - Cat. No. 400000 • 8-Isoprostane Affinity Column - Cat. No. 416358 • 8-Isoprostane Affinity Sorbent - Cat. No. 416359 • 8-Isoprostane EIA Kit - Cat. No. 516351 • 8-Isoprostane Affinity Purification Kit (20 ml) - Cat. No. 516358

NOTES

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