Product Information

8-Isoprostane Affinity Column

Item No. 401111

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 is available for purchase from Cayman (Item No. 400000).

Product Description

The column contains 0.5 ml affinity sorbent (mouse anti-8-isoprostane antibody covalently bound to Sepharose 4B) and has a binding capacity of 5 ng 8-isoprostane (10 ng 8-isoprostance/ml sorbent). This column will be stable for at least two years when stored at 4°C. Storing the columns frozen is not recommended. Be certain that the column is stored in an upright position.

There are two general uses of this affinity column. The first, and most common, is for rapid purification of 8-isoprostane from biological samples for subsequent analysis for 8-isoprostane by EIA. The second use is for removal of 8-isoprostane from biological samples, particularly when 8-isoprostane interferes with assaying a different target molecule.

NOTE: This affinity columns 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 columns are intended for single use only. We do not recommend reuse.

Sample Preparation

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 centrifuged briefly and diluted 1:5 with Column Buffer (see below).

Reagent Preparation

Prepare the following reagents for use with the affinity sorbent.

1. **Eicosanoid Affinity Column Buffer**
   Prepare a 0.1 M phosphate buffer solution by combining 13.3 g potassium phosphate (dibasic) 3.22 g potassium phosphate (monobasic) 0.5 g sodium azide, and 29.2 g sodium chloride. Dilute to a total volume of 1.0 liter with UltraPure Water. The pH of this buffer will be 7.4. This buffer may be purchased as a 5X concentrated buffer (Item No. 400220).
2. **Eicosanoid Affinity Column Elution Solution**
   Prepare a solution containing 95% absolute ethanol and 5% UltraPure Water. This solution may be purchased (Item No. 400230).
3. **EIA Buffer**
   Combine 13.3 g potassium phosphate (dibasic) 3.2 g potassium phosphate (monobasic) 23.4 g sodium chloride, 370 mg tetrasodium EDTA, 100 mg sodium azide, and 1 g bovine serum albumin (Sigma A7030 or equivalent) in a final volume of 1,000 ml. Stir at room temperature until completely dissolved. The pH will be 7.0-7.4. This buffer may be purchased as a 10X concentrated buffer (Item No. 400060).

Cayman Chemical

Mailing address
1180 E. Ellsworth Road
Ann Arbor, MI
48108 USA

Phone
(800) 364-9897
(734) 971-3335

Fax
(734) 971-3640

E-Mail
custserv@caymanchem.com

Web
www.caymanchem.com

Product Information

Protocol for Purification or Removal of 8-Isoprostane

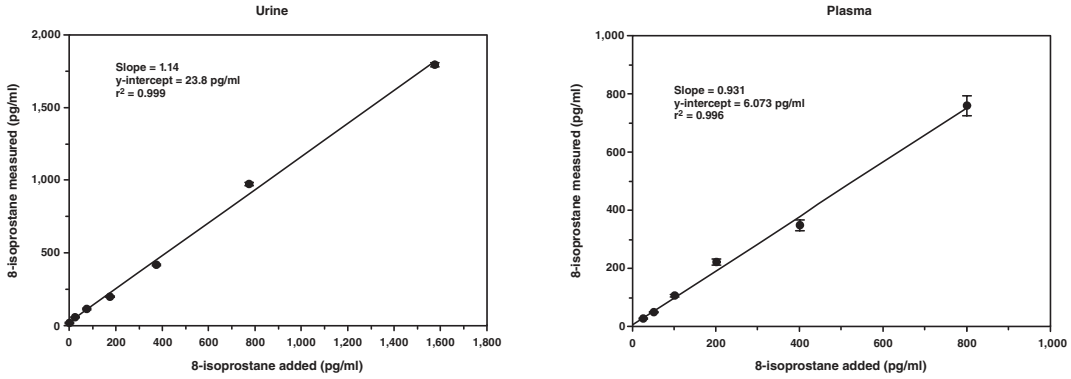
1. Remove the top cap from the column and then gently remove the bottom plug. Be certain to remove the top cap first to avoid air bubbles being drawn into the settled sorbent.
2. Allow the storage solution to pass through the sorbent and then wash the column with 2 ml of Column Buffer. Repeat wash.
3. Apply the sample to the column and allow the entire sample to pass through the sorbent.
4. Wash the column with 2 ml Column Buffer, followed by 2 ml UltraPure Water. Allow all the water to pass through the sorbent. Discard both of these washes.
5. 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 sorbent. Repeat with an additional 2 ml Elution Solution. If the analysis cannot be performed immediately, store the sample in the Elution Solution at -80°C; it will be stable for at least one year.
6. Evaporate the Elution Solution to dryness either by vacuum centrifugation or under a stream of dry nitrogen. It is important that all the organic solvent be removed as even trace quantities may adversely affect an immunoassay.
7. Immediately dissolve the 8-isoprostane in buffer or solvent appropriate for your application. If you are assaying the sample with one of our 8-Isoprostane EIA Kits (Item No. 516351 or Item No. 516360), 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.

Recovery

Urine and plasma recoveries average >90%.

Sample Data

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



Related Product

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