Product Information

8-Isoprostane Affinity Sorbent

Item No. 401113

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

This vial contains 1 ml of an 8-isoprostane (8-iso $PGF_{2\alpha}$) affinity sorbent (mouse anti-8-isoprostane antibody covalently bound to Sepharose 4B) supplied in Eicosanoid Affinity Column Buffer. This sorbent is stable for at least two years if stored at 4°C. Storing the sorbent frozen is not recommended. One milliliter of sorbent is capable of binding approximately 10 ng of 8-isoprostane.

Cayman Chemical also offers prepared 8-Isoprostane Affinity Columns (Item No. 401111) containing 0.5 ml Affinity Sorbent.

There are two general uses of this sorbent. 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 purpose is for removal of 8-isoprostane from biological samples, particularly when 8-isoprostane interferes with assaying a different target molecule.

NOTE: This affinity sorbent 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 sorbent is intended for single use only. We do not recommend reuse.

Sample Preparation

All samples must be free of particulates and precipitates and be at approximately neutral pH (6.5-7.5). Urine samples should be centrifuged briefly to remove sediment. Plasma samples should be centrifuged briefly and diluted 1:5 with Column Buffer.

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).

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Protocol for Purification or Removal of 8-Isoprostane

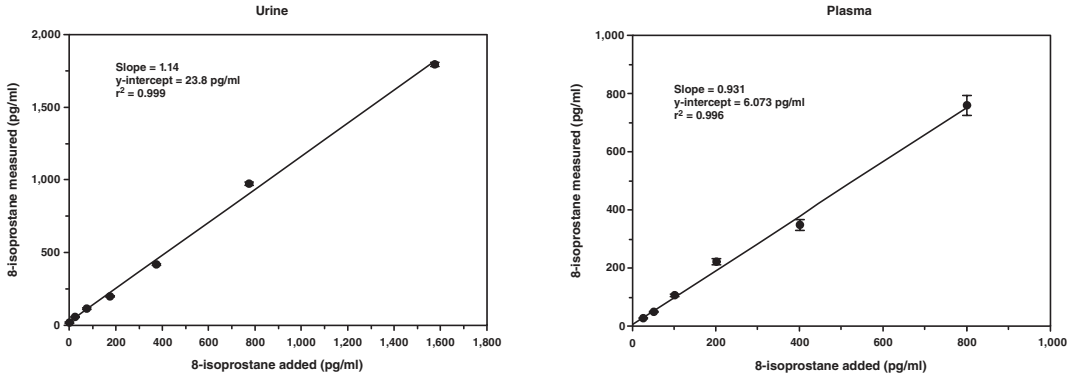
1. Prior to use wash the sorbent two times with 2-4 volumes of column buffer as follows. Transfer the sorbent to a microfuge or similar tube and briefly centrifuge at 1500 x g to sediment the sorbent. Remove the supernatant with a pipette and discard. Add 2-4 volumes of Column Buffer to the sorbent, mix gently, repeat centrifugation and removal of supernatant. Repeat once. Resuspend the sorbent with approximately an equivalent volume of Column Buffer.
2. Aliquot 1 ml of sample in to a 1.5 ml microfuge or similar tube. Larger samples can be used. We recommend using plastic conical centrifuge tubes to facilitate collection of the sorbent by centrifugation. When using large volumes of sample, the amount of sorbent and the incubation time required for binding the 8-isoprostane may need to be increased accordingly from the amounts and times described below.
3. Gently mix the washed sorbent prior to use. Add 50-100 µl of sorbent to the sample and mix gently for 30-60 minutes. The amount of sorbent needed will depend on the amount and concentration of the sample being analyzed. For example, since 1 ml of sorbent will bind 10 ng of 8-isoprostane, 50 µl of sorbent is capable of binding 500 pg of 8-isoprostane. Be sure to add enough sorbent to bind the amount of 8-isoprostane you expect your sample will contain.
4. Briefly centrifuge the sample at 1,500 x g to sediment the sorbent.
5. Carefully remove the supernatant with a pipette or by decanting. Care must be taken to retain all the sorbent as it contains bound 8-isoprostane. The supernatant solution will be devoid of 8-isoprostane.
6. Wash the sorbent once with 1 ml of Eicosanoid Column Affinity Buffer, centrifuge, and carefully remove the supernatant solution.
7. Wash the sorbent once with 1 ml of UltraPure water, centrifuge and carefully remove the supernatant solution.
8. To elute the 8-isoprostane from the sorbent, resuspend the sorbent pellet in 0.5 ml of Elution Solution. Vortex briefly.
9. Centrifuge to sediment the sorbent and carefully transfer the Elution Solution containing 8-isoprostane to a clean tube. Repeat this elution a second time combining the elution into one tube. If the analysis cannot be performed at once, store the sample in the Elution Solution at -80°C; it will be stable for at least one year.
10. Evaporate to dryness under nitrogen or vacuum centrifugation. Resuspend in solvent of choice and analyze.

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

For a list of related products please visit: www.caymanchem.com/catalog/401113

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