

Product Information



8-Isoprostane Affinity Sorbent

Item No. 10010365 • 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 be purchased (Item No. 400000).

Laboratory Procedures

This vial contains 1 ml of an 8-isoprostane (8-*iso* PGF_{2α}) affinity sorbent (mouse 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/ml of 8-isoprostane. (Cayman Chemical also offers prepared 8-Isoprostane Affinity Columns, Item Nos. 400058 and 400059).

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 of a different target molecule. Both purposes can be accomplished in either batch (as described below) or column formats.

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 K₂HPO₄, 3.22 g KH₂PO₄, 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

Prepare a 1.0 M phosphate buffer solution by combining 133 g K₂HPO₄ and 32.15 g KH₂PO₄ 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 be purchased as a 10X concentrated buffer (Item No. 400060).

"Batch" Format for Purification or Removal of 8-isoprostane

1. Prior to use wash the sorbent two times with 2-4 volumes 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 three to five times 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.

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.

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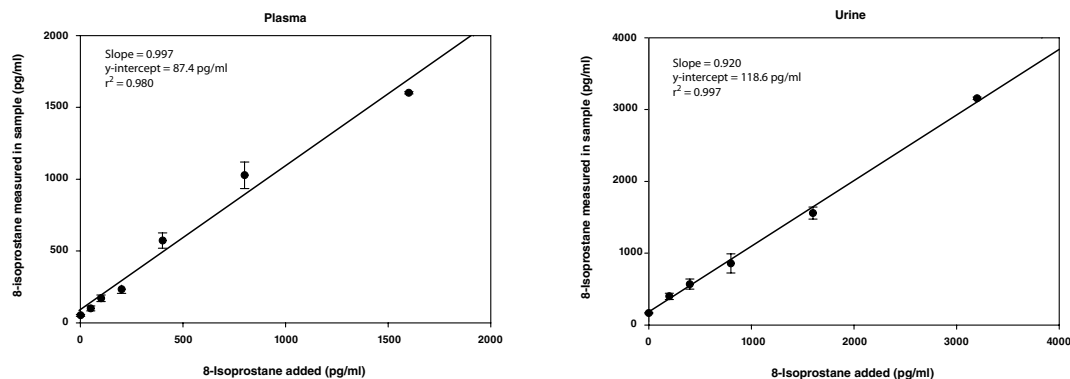
- Carefully remove the supernatant with a pipette or by decanting. Care must be taken to retain all the sorbent as it contains bound 8-isoprostane. The supernatant solution will be devoid of 8-isoprostane.
- Wash the sorbent once with 1 ml of Eicosanoid Column Affinity Buffer, centrifuge, and carefully remove the supernatant solution.
- Wash the sorbent once with 1 ml of UltraPure water, centrifuge and carefully remove the supernatant solution.
- To elute the 8-isoprostane from the sorbent, resuspend the sorbent pellet in 0.5 ml of Elution Solution. Vortex briefly.
- 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. (Do not discard the sorbent as it can be regenerated according to the protocol described below.)
- Evaporate to dryness under nitrogen or vacuum centrifugation. Resuspend in solvent of choice and analyze. If the analysis cannot be performed at once, store the eluted samples at -80°C ; they will be stable for at least one year.

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 sorbent in column format. Urine or plasma was spiked with the indicated amounts of 8-isoprostane and purifications were performed for each concentration. The recovery was greater than 90%.



Regenerating the sorbent

After the elutions have been performed to release bound 8-isoprostane, as described above, the sorbent will be devoid of bound 8-isoprostane and can be equilibrated for additional use. Equilibrate the sorbent by washing with five volumes of water followed by five volumes of Column Buffer. The equilibration can be performed in a plastic conical centrifuge tube. If several milliliters of sorbent are being equilibrated a small funnel with a fritted filter can be used. Store the sorbent in two volumes of Column Buffer. For plasma samples, the sorbent can be used up to four times when purifying 200 μl of plasma per 0.5 ml of sorbent. For urine samples, each aliquot of sorbent should be limited to a single use. Each regeneration cycle decreases the sorbent binding capacity, so fresh and regenerated sorbents are not strictly comparable. We urge you to use identical sorbent lots for each study or data set.

If the sorbent has been used to remove 8-isoprostane from the sample of interest, 8-isoprostane will be bound to the sorbent. To regenerate the sorbent, suspend several milliliters of spent sorbent in 10 ml of UltraPure water in a small funnel with a fritted filter. Wash with ten volumes Elution Solution and ten volumes water. Re-equilibrate the sorbent in Column Buffer. Store the sorbent in two volumes of Column Buffer. Smaller amounts of sorbent can be regenerated by performing the procedure in a 15 ml conical plastic centrifuge tube. The capacity of the sorbent will decrease with subsequent use.

Specificity

8-Isoprostane	100%	8,12- <i>epi</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>epi</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

For a list of related products please visit: www.caymanchem.com/catalog/10010365

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Product Information



Analytical Data Sheet

8-Isoprostane Affinity Sorbent

Item No. 10010365 • Lot No. XXXXX

Date of Manufacture: XXXXX

Assay Conditions

1. Eicosanoid Affinity Column Buffer (Item 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 (Item 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 (Item No. 400230).
 - g. The Elution Solution was evaporated under a stream nitrogen and reconstituted in 2 ml EIA buffer (Item 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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