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Lieferung & Zahlungsart

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- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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PRODUCT INFORMATION

Cysteinyl Leukotriene Affinity Sorbent

Item No. 400396

Overview and Properties

Contents: This vial contains 5 ml of cysteinyl leukotriene affinity sorbent.
Storage: 4°C (as supplied)
Stability: As supplied, 1 year from the QC date provided on the Certificate of Analysis, when stored properly

Procedures

This vial contains 5 ml of cysteinyl leukotriene (cysLT) affinity sorbent (mouse monoclonal anti-LTC_4/LTD_4/LTE_4 covalently bound to Sepharose 4B) supplied as a 50:50 mixture in in Eicosanoid Affinity Column Buffer. This sorbent is stable for at least two years if stored at 4°C. Do NOT freeze. The sorbent has a binding capacity of 10 ng CysLT per ml of sorbent determined using LTE_4.

Sorbent Preparation

Wash the sorbent with 2 to 4 volumes of Eicosanoid Affinity Column Buffer (Item No. 400220). Briefly centrifuge at 500 x g to sediment the sorbent. Repeat wash. Gently mix the sorbent prior to use by vortexing or inversion to completely resuspend the sorbent in the buffer. Best results are obtained when a moderate excess of the sorbent is used to bind the CysLTs from biological samples. We recommend using 50 µl-200 µl of the resuspended sorbent per 1 ml of sample.

Eicosanoid Affinity Column Buffer Preparation

Prepare a 0.1 M phosphate buffer solution by combining 13.3 g K_2HPO_4, 3.22 g KH_2PO_4, 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 7.0. This buffer may also be purchased as a 5X concentrated buffer (Item No. 400220).

Sample Preparation

This sorbent has been validated with the LTE_4 and CysLT Express EIA Kits (Item No. 520411 and 10009291, respectively) for human urinary cysLT extraction. Purification of other sample matrices have not been fully validated.

Urine

When known amounts of LTE_4 are spiked into human urine samples it was found that 200 µl of sorbent is sufficient to extract between 20 pg and 2 ng LTE_4 from 1 ml of normal human urine.

Purification Protocol

1. Aliquot 1 ml of sample to a 1.5 ml microfuge or similar tube. Larger samples can be used. It is important to also include a blank extract (sorbent incubated in buffer alone). We recommend using plastic conical centrifuge tubes to facilitate collection of the sorbent by centrifugation. When using larger volumes of sample, the amount of sorbent and incubation time may need to be increased.
2. Gently mix the sorbent prior to use to resuspend. Add 50-200 µl of resuspended sorbent mixture to each 1 ml sample (or blank) and mix gently for 30-60 minutes. The amount of sorbent needed will depend on the concentration of CysLT in the sample.
3. Briefly centrifuge the samples at 500 x g to sediment the sorbent.
4. Carefully remove the supernatant by aspirating with a pipette. Care must be taken to retain all the sorbent as this contains the bound CysLT.

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5. Wash the sorbent once with 1 ml Column Buffer, centrifuge, and carefully remove and discard the supernatant.
6. Resuspend the sorbent in 0.5 ml ice-cold methanol. Vortex briefly.
7. Centrifuge to sediment the sorbent and carefully remove the methanol and transfer to a clean tube. DO NOT DISCARD the methanol as this contains the eluted CysLT. Repeat Steps 6 and 7.
8. Combine the methanol eluates and evaporate to dryness under nitrogen or vacuum. If the analysis cannot be performed at once, store the methanol eluates at -80°C; they will be stable for at least six months.
9. Immediately dissolve the CysLT in the buffer or solvent appropriate for your application. If you are assaying the sample with one of our EIA kits (Cysteinyl Leukotriene EIA Kit - Item No. 500390; Cysteinyl Leukotriene Express EIA Kit - Item No. 10009291; Leukotriene E4 EIA Kit - Item No. 520411), dissolve the sample in the buffer or solvent recommended in the instructions provided with the kit. The amount of buffer depends on the original volume and the expected concentration of CysLT in the sample. A dilute sample may be concentrated by dissolving the residue in a smaller volume of buffer than the original sample volume. *NOTE: Remember to also subtract the value of the blank (i.e., buffer extracted sample) from all sample values in the final analysis.*

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