Product Information

Cysteinyl Leukotriene Affinity Column (4 ml)

Item No. 400068

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 (Item No. 400000).

Laboratory Procedures

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

1. **Cysteinyl Leukotriene Affinity Column**
   This column contains 0.5 ml sorbent and and has a binding capacity of 5 ng CysLT. 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. Wash the column with 2 ml of column buffer. Repeat wash. The column is now ready to use.
2. **Eicosanoid Affinity Column Buffer**
   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 7.4. This buffer may also be purchased as a 5X concentrated buffer (Item No. 400220).
3. **Methanol**
   Place an aliquot of 100% methanol on ice.

Purification Protocol

1. 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 (a dilution of 1:2 is recommended for best recovery). 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.
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. Elute the cysLT from the column by adding 2 ml methanol and allowing it to pass through the packing material. If the analysis cannot be performed at once, store methanol fractions at -80°C; they will be stable for at least one year.
4. Evaporate the methanol 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 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.

Related Product

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