20-HETE Immunoaffinity Column for one-step purification

Catalog Number: 20H33
Store at 4°C
FOR RESEARCH USE ONLY

Overview

The 20-HETE immunoaffinity column is used as a one-step purification process. Each column has a binding capacity of 1 microgram 20-HETE.

Materials Provided with Kit

- **10 microspin columns:** 10 columns containing 100 ul of immunoaffinity resin each in PBS buffer containing 0.02% sodium azide.
- **20 X 2 ml Collection Tubes**
- **Washing Buffer:** 50 ml
- **10X Sample Dilution Buffer:** 25 ml. Dilute to 1X Sample Dilution Buffer with high purity water

Additional Required Materials

- **Elution Solution:** Acetone : high purity water (90 : 10)

Column Preparation

Each column comes with a breakable bottom stem. Before snapping off the bottom of the tube, shake the column a few times ensure uniform mixing of resin in the column. The breaking point of the column is at the indented circumference position below the column. Before snapping the breakable point, shake the column a few times to ensure uniform mixing of resin with the buffer. Once the bottom stem is snapped off, it can be inverted and used as a stopper for the column. Partially unscrew the cap. (This procedure of un capping should be followed for all subsequent spins). Remove the stopper from the column. Place the column into a 2ml collection tube. Then centrifuge the column at 100g for 2 minutes. Discard the flow through collected at the bottom of the tube. The sample should then be added immediately to the column to prevent drying of the resin.

Sample Incubation: For each 100 uL resin column a sample volume of 100 uL is recommended (maximum load = 400 uL). After the sample is added to the column, incubate with resin for 30 minutes at room temperature. Periodic mixing of column through either careful tapping or through use of a pipette will enhance the incubation.

Wash: After incubation, spin the column at 100g for 2 minutes. Discard the flow through. Then add 400 uL wash buffer to column and spin for 1 ½ minutes at 100g. Discard the flow through. Repeat this wash step 4 more times with fresh buffer each time. Discard all flow through. For the

final wash add 400 uL fresh wash buffer and spin at 300 to 400g for 2 minutes. Discard flow through (Please note that a light mixing of wash buffer with resin each time may enhance the wash process). After the final wash step go immediately to the elution step.

Elution: For elution of the column, use a new 2 ml collection tube. To elute the bound 8-isoprstane from the column, add 100 ul of elution solution to the resin and let incubate for 30 seconds with cap on. Then spin at 100g for 1 minute (do **not** discard the flow through as it can be kept in the collection tube during the second elution spin). For the second elution spin add another 100 ul of elution solution to resin and incubate for 30 seconds with cap on. Then spin for 2 minutes at 300 to 400g. Save the combined eluent. Evaporate the eluted product under nitrogen or argon gas until dry*. If the analysis cannot be performed at once, store the eluent at -80 °C. Before the Elisa assay, dissolve isolated 20-HETE in 20 uL ethanol and mix well. If it is difficult to estimate the concentration of isolated 20-HETE, it is recommended to serially dilute the sample with 1 X Sample dilution buffer (1:10, 1:100 1:1000 etc.) until the results fall into the linear portion of the standard curve (between 10 ng/ml and 0.01 ng/ml).

*Drying until a volume of approx. 20 ul should produce comparable results as directed by the above procedure. This sample should however be used right away for analysis. Although nitrogen or argon is recommended, a speed vacuum system can be used for drying.