14,15-DHET Immunoaffinity Column

Catalog Number: **DH31**
Store at 4°C.
FOR RESEARCH USE ONLY

Overview

The 14,15-DHET immunoaffinity column is used as a one-step purification process.

Storage and Stability

Upon receipt of this product please store at 4°C. Do not remove from refrigeration more than necessary.

Materials Provided

- **10 microspin columns**: 10 columns containing 100 µL of immunoaffinity resin each in PBS buffer containing 0.01% 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 ultra pure water.

Additional Required Materials (Not Provided)

- **Elution Solution**: acetone : ultra pure water (90:10)

Column Preparation

Each column comes with a breakable bottom stem. The break point is the indented circumference positioned below the column. Once the bottom stem is snapped off, it can be used as a stopper once it is reversed and placed into the break point on the bottom of the column. Before snapping the breakable point, shake the column a few times to ensure uniform mixing of resin with the buffer. Unscrew cap only partially. (This procedure of capping should be followed for all subsequent spins.) Remove the bottom breakable stem portion. Connect the column to a 2 mL collection tube. Then centrifuge the column at 1000 rpm for two minutes. Discard flow through (will be in 2 mL collection tube). The sample should then be added immediately to the column to prevent dryness of the resin.

Sample Incubation

For each 100 µL resin column a sample volume of 100 µL is recommended. After the sample is added to the column, incubate with resin for 30 minutes at room temperature. Periodic mixing of the column, through either careful tapping or through use of a pipette (as a mixer), will enhance the incubation.

Wash (7 washes)

After incubation, spin the column at 1000 rpm for two minutes. Discard the flow through (will be in the 2 mL collection tube). For the first wash add 400 μL of wash buffer to the column and spin for 1.5 minutes at 1000 rpm. Discard the flow through. Repeat this wash step four more times (washes 2-5) using fresh wash buffer each time. Discard all flow throughs. For the sixth and seventh washes add 400 μL of fresh wash buffer and spin at 1750-2000 rpm for two minutes. Discard the flow throughs each time. (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 the elution use a new 2 mL collection tube. To elute the bound 14,15-DHET from the column add 100 μL of elution solution to resin and let it incubate for 30 seconds with the cap on. Then spin at 1000 rpm for one 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 μL of elution solution to the resin and incubate for 30 seconds with the cap on. Then spin for two minutes at 1750-2000 rpm. Save the combined eluate. Evaporate the eluted product under argon gas until dry.* If the analysis cannot be performed at once, store the eluant at -80℃. Before the ELISA assay, dissolve the isolated DHET in 20 μL of ethanol and mix well. If a rough estimate of the concentration of isolated DHET is unknown, it is recommended to dilute the sample with 1X Sample Dilution Buffer at different magnitudes (such as 1:10, 1:100, 1:1000, etc.) until the results fall into the readable portion of the standard curve (between 10 ng/mL and 0.01 ng/mL).

*Drying until a volume of approximately 20 μL should produce comparable results as directed by the above procedure. This sample should, however, be used right away for analysis. Although argon is recommended, a fats working speed vacuum system can be used in place of argon.

Detroit R&D, Inc.

Metro Center For High Technology Bldg. (MCHT)
2727 Second Ave. Suite 4113
Detroit, MI 48201

Phone: 313.961.1606
Fax: 313.963.7130
E-mail: info@detroitrandd.com
www.DetroitRandD.com