

Extraction Procedure for Leukotrienes and 15-Epi Lipoxin A₄

Includes: Leukotriene B₄, Leukotriene C₄, Leukotriene C₄/D₄/E₄, and 15-Epi Lipoxin A₄

Cat #'s: EA 35, EA 38, EA 39, and EA 46

Materials Needed:

1. 1 N HCl
2. C₁₈ Sep-Pak® Columns (Waters® Corporation)
3. Ethanol
4. Deionized Water
5. Petroleum Ether
6. Methyl Formate
7. Nitrogen Gas

Procedure:

1. For 1 mL plasma or urine: acidify to pH 3.5 with 1 N HCl (1 mL of plasma requires about 150 µL of 1 N HCl).
For tissue: Homogenize the tissue in ethanol (5 mL/g) and centrifuge to obtain supernatant. Dilute 1 mL of the supernatant with 5 mL of water and acidify to pH 3.5 with 1 N HCl.
2. Precondition the C₁₈ Sep-Pak® light column (Waters® Corporation) by washing the column with 2 mL of ethanol followed by 2 mL of water.
3. Apply the above sample into the column and adjust the flow rate to 1 mL per minute. Reducing the flow rate to 0.5 mL per minute may increase extraction efficiencies. Some samples may clog the column. These samples may be diluted 1:5 in water to improve the flow rate.
4. Wash the column with 1 mL of water followed by 1 mL of petroleum ether.
5. Elute eicosanoid with 2 mL of methyl formate.
6. Evaporate the methyl formate with a stream of nitrogen gas.