

## Extraction Procedure for Lipoxin A<sub>4</sub>

Includes: Lipoxin A<sub>4</sub>

Cat #: EA 45

### Materials Needed:

1. Methanol
2. Deionized Water
3. 1 N HCl
4. Methanol
5. C<sub>18</sub> Sep-Pak® Columns (Waters® Corporation)
6. Hexane
7. Methyl Formate
8. Nitrogen Gas

### Procedure:

1. Dilute 100 µL of sample with 200 µL of methanol, and then dilute the previous volume with 1.5 mL of water.
2. For 1 mL sample: 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 methanol (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.
3. Precondition the C<sub>18</sub> Sep-Pak® light column (Waters® Corporation) by washing the column with 2 mL of methanol followed by 2 mL of water.
4. Apply the above sample into the column and wash the column with 5 mL of water followed by 5 mL of hexane.
5. Elute Lipoxin A<sub>4</sub> with 2 mL of methyl formate.
6. Evaporate the methyl formate with a stream of nitrogen gas.