DetectX®

Eicosanoid Sample Extraction Protocol

For use with our DetectX® Eicosanoid Immunoassay Kits

ARBOR ASSAYS®

INTRODUCTION

For extraction of prostaglandins, thromboxanes and leukotrienes from biological samples we recommend an organic phase extraction. Listed below is our modification of standard protocols. A useful resource can be found online at lipidlibrary.aocs.org/lipid-analysis/selected-topics-in-the-analysis-of-lipids/solid-phase-extraction-columns-in-the-analysis-of-lipids. The protocol uses chemically-bonded octadecylsilyl ("ODS" or "C18") solid phase cartridges to bind the bioactive lipid from the biological sample followed by elution.

MATERIALS NEEDED

- C18 Cartridges, such as Bond-Elut™, Sep-Pak™ or similar
- Eicosanoid standard to allow extraction efficiency determination
- 2M hydrochloric acid, deionized water, ethanol, hexane and ethyl acetate

All biological samples should have a cyclooxygenase inhibitor, such as indomethacin or meclofenamic acid at 10-15 µM, added immediately after collection. For aqueous tissue homogenates, serum or plasma, we suggest adding ethanol to a concentration of 15% prior to being acidified and passage through the ODS/C18 cartridge. We suggest checking the efficiency of extraction in order to adjust final sample concentrations if necessary. First identify the approximate 50% binding point of the assay from the kit insert. Using the kit standard and Assay Buffer (AB) prepare a High Concentration Spike (HCS) at 10 times the concentration of the 50% B/B0 binding point. Prepare the following Control samples of equal volume using this HCS solution:

Spiked Assay Buffer (SAB):	An aliquot of AB spiked with 5% by volume of the HCS.
Unspiked Sample (US):	Untreated sample spiked with 5% by volume of AB.
Spiked sample (SS):	Sample spiked with 5% by volume of the HCS.

Extract samples and US and SS Controls as described in steps 1-6. **Do not extract SAB Control.**

1. Acidify the serum, plasma, urine or tissue homogenate samples by addition of 2M hydrochloric acid to pH of 3.5. Approximately 50 µL of hydrochloric acid will be needed per mL of plasma. Allow to sit at 4°C for 15 minutes. Centrifuge samples in a microcentrifuge for 2 minutes to remove any precipitate.
2. Prepare the ODS/C18 reverse phase columns by washing with 20mL of ethanol followed by 20mL of deionized water.
3. Apply the samples to the columns under a slight positive pressure to obtain a flow rate of about 0.5 mL/minute. Wash the column with 10 mL of water, followed by 10 mL of water:ethanol (85:15), and finally 10 mL hexane. Elute the samples from the column by addition of 10 mL ethyl acetate.
4. **If analysis is to be carried out immediately**, evaporate samples in a centrifugal vacuum evaporator (SpeedVac™) or under a stream of nitrogen. Add at least 250 µL of Assay Buffer to the dried samples. Bring the US and SS Controls up in the original volume of AB. Vortex well then allow to sit for five minutes at room temperature. Repeat vortexing twice more.

   If analysis is to be delayed, store samples as the eluted ethyl acetate solutions at -80 °C until the immunoassay is to be run. Evaporate the organic solvent and reconstitute as described above prior to running assay.
5. 5. Determine the eicosanoid content of the samples, SAB, US and SS Controls by taking the Assay Buffer solutions and running them in the appropriate Arbor Assays kit. For example, Prostaglandin E_2 samples in the range of 1,000 to 31.25 pg/mL should be run in either the regular sensitivity PGE_2 kit, K018-H1 (one plate) or K018-H5 (five plates). For more dilute samples use the High Sensitivity PGE_2 kit, K018-HX1 (one plate) or K018-HX5 (five plates).

6. Determine the extraction efficiency by comparing the immunoassay concentration of the eicosanoid measured in the extracted Controls (US and SS) to the unextracted Control (SAB).

   Immunoassay Concentration in Extracted US = EUS
   Immunoassay Concentration in Extracted SS = ESS

   The efficiency = $\frac{\text{ESS} - \text{EUS}}{\text{SAB}}$