DetectX™

Steroid Tissue Extraction Protocol

For our DetectX™ Steroid Immunoassay Kits

ARBOR ASSAYS®

INTRODUCTION:

To extract steroids from tissue samples we recommend an organic phase extraction. Listed below is our modification of standard protocols. We use acetonitrile to solubilize the steroid and hexane to remove fat and lipid that may be present in the sample. We recommend centrifugal vacuum devices (i.e., a SpeedVac™) to remove the solvent completely and safely. We also recommend the use of ethanol as a means to completely solubilize the dried steroid because certain steroids have limited aqueous solubility.

MATERIALS NEEDED:

* Steroid standard to allow extraction efficiency determination
* ACS Grade Acetonitrile, Sigma 360457 or similar
* ACS Grade Hexane, Sigma 32293 or similar
* 50 mL Polypropylene Centrifuge tubes
* Glass test tubes for evaporation

PROCEDURE:

Ensure that the sample is as free from debris and as dry as possible. Weigh 50 mg of samples in a 50 mL centrifuge tubes. Add sufficient steroid to a portion of one sample (Control Sample) to check for efficiency of extraction. Add 15 mL of acetonitrile and homogenize using a hand held or bead homogenizer. Centrifuge the mixture at 10,160 x g for 10 min at 4°C and carefully remove the supernatant to a clean tube. Add 30 ml of hexane to the supernatant and shake the tube vigorously for 5 minutes. Separate the lower heavier layer of acetonitrile to a clean glass tube and discard the hexane layer.

1. Evaporate the acetonitrile supernatant solution to dryness in a SpeedVac or under nitrogen. Keep dried extracted samples frozen < -20°C in a desiccator.

2. Dissolve extracted sample with 100µL ethanol, followed by at least 400µL of the kit specific Assay Buffer. Vortex well and allow to sit 5 minutes at room temperature. Vortex and sit for 5 minutes twice more to ensure complete steroid solubility. **For immunoassays ethanol content in the sample volume should be below 5%.** Dilute the ethanol:Assay Buffer mixture ≥ 1:10 with kit specific Assay Buffer, or as directed in the kit manual.

3. Run reconstituted diluted samples in assay immediately according to insert directions.

4. Extraction efficiency is determined by comparing the concentration of the steroid measured in the extracted Control sample with the known concentration of steroid spike (preferably measured or theoretical) and the steroid concentration determined in the unspiked sample after extraction.

$$\% \text{ Efficiency} = \frac{(\text{Steroid in Control Sample} - \text{Steroid in Unspiked Sample})}{\text{Steroid Spike Concentration}} \times 100$$

General reference: http://link.springer.com/chapter/10.1023%2Fb135931_3