DetectX™

Steroid Solid Extraction Protocol

For our DetectX™ Steroid Immunoassay Kits

ARBOR ASSAYS®

INTRODUCTION:

To extract steroids from non-liquid matrices, such as dried solids or other organic matter, we recommend an organic phase extraction. Listed below is our modification of standard protocols. We use ethanol or ethyl acetate as a safer alternative to diethyl ether or methylene chloride. 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. The protocol uses ethanol or ethyl acetate to extract the organic soluble steroid. The organic layer is separated and stored.

MATERIALS NEEDED:

* Steroid standard to allow extraction efficiency determination
* ACS Grade Ethanol (or Ethyl Acetate)
* Glass test tubes

PROCEDURE:

Ensure that the sample is completely dry and powder the sample to improve extraction efficiency. Remove any large particles, such as grass, if possible. We suggest checking the efficiency of extraction by preparing a steroid solution of known concentration in the kit Assay Buffer (AB). Spike one aliquot of your sample with a volume of the steroid solution in AB (Control Spike) and one aliquot of sample with the same volume of AB (Control Sample). Extract samples and both Controls with Ethanol or Ethyl Acetate as described below.

1. Weigh out ≥ 0.2 gm of dried fecal solid into a tube.

2. Add 1 mL of Ethanol (or Ethyl Acetate) for every 0.1 gm of solid.

3. Shake vigorously for at least 30 minutes.

4. Centrifuge samples at 5,000 rpm for 15 minutes. Transfer measured volume of supernatant to a clean tube for evaporation.

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

6. Dissolve extracted sample with 100µL ethanol, followed by at least 400µL AB. 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 well should be below 5%.** Dilute the ethanol:AB mixture ≥ 1:10 with AB, or as directed in the kit manual.

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

8. Determine the extraction efficiency by comparing the concentration of the steroid measured in the extracted Control (Control Spike - Control Sample) with the concentration of steroid before extraction.

Note: In step 5 if only a portion of the organic solvent is being evaporated, ensure final amounts of measured steroid per gm solid accounts for volume of solution evaporated.