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. 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 recovery. 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). See Extraction Efficiency section below. 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. (0.1 gm fecal solid/mL)

3. Shake vigorously for at least 30 minutes.

4. Centrifuge samples at 5,000 rpm for 15 minutes. Reserve supernatant in a clean tube. This material is able to be stored at ≤ -20°C for at least a month if properly sealed.

5. Transfer a measured volume of supernatant (Evaporation Vol.) into a clean tube and evaporate 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 (Reconstitution Vol.). Vortex well and allow to sit 5 minutes at room temperature. Vortex and let 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 with AB as directed in the kit manual.

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

SAMPLE ANALYTE CONCENTRATION CALCULATION:

Assay Concentration (ie. pg/mL) x Assay Dilution Factor x Reconstitution Vol (mL) ÷ Evaporation Vol (mL) ÷ 0.1g fecal solid/mL = Analyte unit (i.e., pg/g) fecal solid.

EXTRACTION EFFICIENCY:

To determine efficiency, one sample will be prepared twice; once with a known amount of analyte added to it (spike) and once with an equivalent volume of AB added to it (unspiked) to represent the volume of the spike. The two samples are processed along with the other extracted samples. The Extraction Efficiency is calculated with the following formula:

(Measured Spiked conc. - Measured Unspiked Conc.)/Concentration of Spike = Efficiency

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