DetectX®

Steroid Solid Extraction Protocol

For our DetectX® Steroid Immunoassay Kits

ARBOR ASSAYS®

INTRODUCTION

We recommend an organic phase extraction to extract steroids from non-liquid matrices such as dried solids or other organic matter. We use ethanol or ethyl acetate as safer alternatives to diethyl ether or methylene chloride. We recommend centrifugal vacuum devices (i.e., a SpeedVacTM) to remove the solvent completely and safely after extraction. Alternatively, the organic layer can be separated and stored for use at a later date. We also recommend using ethanol to completely solubilize the dried extracted steroid because steroids typically have limited aqueous solubility.

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). Please refer to Extraction Efficiency section below for details. Extract samples and Extraction Efficiency Controls with ethanol or ethyl acetate as follows:

1. Weigh out ≥ 0.2 gm of dried fecal solid into a tube. Samples can be dried by passive drying, gentle heating (≤ 60°C), or freeze-drying (lyophilization).
2. Add 1 mL of ethanol (or ethyl acetate) for every 0.1 gm of solid (0.1 gm fecal solid/mL) and seal.
3. Shake vigorously for at least 30 minutes.
4. Centrifuge samples at 5,000 rpm for 15 minutes at 4°C. Reserve supernatant in a clean tube. This material can be stored at ≤ -20°C for at least a month if properly sealed.

   Note: Samples containing low levels of analyte can be concentrated by drying down extract and resuspending in a reduced volume of Assay Buffer. Samples extracted with ethyl acetate must be dried down completely to remove the solvent prior to addition to aqueous buffer. Transfer a measured volume of supernatant from step 4 (Evaporation Vol.) into a clean tube and evaporate to dryness in a SpeedVac or under nitrogen. Dried, extracted samples can be stored frozen ≤ -20°C in a desiccator. Dissolve dried extracted sample with ethanol (e.g., 100µL) and proceed with step 5.

5. **These are general guidelines, please adjust volumes as required.** Add 100µL of extracted sample supernatant from step 4 into a minimum volume of 400µL AB (Total Reconstitution Vol. = 100µL+400µL). Vortex well and allow to rest 5 minutes at room temperature. Vortex and let rest for 5 minutes twice more to ensure complete steroid solubility. **For immunoassays, the ethanol content in the assay typically must be ≤ 5%. This will require additional dilution into Assay Buffer.** It is important to verify the assay's maximum ethanol content tolerance as described under the "sample preparation" section of the kit manual.
6. Run reconstituted diluted samples in assay immediately according to insert directions.

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SAMPLE ANALYTE CONCENTRATION CALCULATION:

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

Example of extraction scenario and subsequent calculations. This is a simplified example only, the volumes described below should be adjusted as required for your specific sample(s).

1. Extract 0.2 gm dried fecal material with 2.0 mL ethanol.
2. Dry the 2.0 mL ethanol extract down to dryness. Re-suspend dried extract in 100uL of Ethanol. (this is a 20x concentration factor of the extract).
3. Add 25µL of the concentrated extract from step 2 to 475µL Assay Buffer. (this is a 20x dilution factor). This preparation will be added to the assay plate as a sample. Add 50µL of this prep to the assay as directed. Run the assay following the instructions included with the kit.
4. If the assay reports 100 pg/mL, multiply this value by 20 to get concentration in concentrated extract (2,000 pg/mL). Divide this value by 20 to get concentration in non-concentrated extract (100 pg/mL).
5. Final adjustments if required: Here, 1.0 mL of solvent was used to extract every 100 mg of fecal material, the amount of analyte present in the starting, dried sample was 100 pg for every 100 mg of starting material (or 10.0 pg/gm of original sample).

   Further adjust this calculated value by the Extraction Efficiency determination if performed. If the Extraction Efficiency is 50%, the adjusted value requires a correction by multiplying by a factor of 2, or by dividing the calculated value by the Extraction Efficiency (assay value ÷ Extraction Efficiency coefficient = actual value). The procedure for determining the Extraction Efficiency is below if required.

EXTRACTION EFFICIENCY

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

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