Steroid Extraction Protocol – Tissue Samples

INTRODUCTION

This protocol serves as a general guideline for extracting steroids from tissue samples, as well as determining extraction efficiency. Specific steps may require adjustment depending on your sample and experimental setup. Always validate your extraction procedure before applying it to high-value samples.

MATERIALS AND EQUIPMENT NEEDED

- DetectX® kit-specific steroid standard for extraction efficiency determination
- DetectX® kit-specific 1X Assay Buffer
- Acetonitrile (≥ 95% purity)
- Hexane (≥ 95% purity)
- Ethanol (≥ 95% purity)
- 50 mL Centrifuge tubes, polypropylene
- Homogenization equipment
- Centrifuge capable of 10,000 x g and an incubation temperature of 4°C
- Analytical balance
- Orbital shaker with test tube adapter
- Centrifugal vacuum device

PROCEDURE

Ensure that the sample is free of blood and completely dry before proceeding.

1. Weigh 50 mg of sample into a 50 mL polypropylene centrifuge tube (Tissue Mass).
2. Add 15 mL of acetonitrile to the centrifuge tube.
3. Homogenize sample completely using homogenization equipment.
4. Centrifuge sample at 10,000 x g for 10 minutes at 4°C. Transfer the analyte-containing supernatant to a clean collection tube.
5. Add 30 mL of hexane to the supernatant and cap the tube.
6. Mix sample using an orbital shaker capable of holding test tubes for 5 minutes.
7. Incubate sample at room temperature without shaking for 5 minutes to allow layers to separate.
8. Transfer the bottom, acetonitrile containing layer to a clean tube. Discard the hexane layer following appropriate chemical safety guidelines.
9. Evaporate the acetonitrile supernatant solution completely using a centrifugal vacuum device. Evaporated samples can be stored at ≤ -20°C in a desiccator.
10. Reconstitute evaporated sample by adding 100 µL of ethanol (Reconstitution Volume).
11. **These are general guidelines. It is important to verify the assay's maximum ethanol content tolerance as described under the "Interference" section of the assay's kit manual. Volumes may need to be adjusted so the sample falls within the standard curve of the assay of interest and adheres to the ethanol tolerance of the assay.** Combine the reconstituted sample from step 10 with a minimum volume of 400 µL 1X Assay Buffer. Briefly vortex and incubate the sample for 5 minutes at room temperature. Repeat vortex and incubation three times to ensure complete steroid solubility. Continue to dilute the sample in 1X Assay Buffer as needed. (Assay Dilution Factor).
12. Run samples in the assay immediately according to the assay protocol (Assay Concentration).

EXTRACTION EFFICIENCY

To obtain a more accurate reading of the analyte concentration of the sample, the extraction efficiency must be calculated. To determine extraction efficiency, one tissue sample will undergo the extraction process twice; once with a known amount of analyte added (Spiked Control) and once with an equivalent volume of 1X Assay Buffer added (Unspiked Control). A third control (Assay Buffer Control) must also be prepared but does not undergo the extraction process. All aliquots must have equivalent volumes (V1) and all spikes must have equivalent volumes (V2). The preparation of these controls is summarized in the table and figure below.

The three controls are then run in the assay according to the assay protocol. Extraction efficiency is calculated using the following formula:

$$\text{Extraction Efficiency (\%)} = \frac{(\text{Spiked Control Concentration} - \text{Unspiked Control Concentration})}{\text{Assay Buffer Control Concentration}} \times 100$$

Extraction Condition	Spiked Control	Unspiked Control	Assay Buffer Control*
Aliquot (V1)	Sample	Sample	1X Assay Buffer
Spike (V2)	Steroid Standard	1X Assay Buffer	Steroid Standard

***The Assay Buffer Control does not undergo the extraction process. Prepare this control immediately prior to assaying samples.**

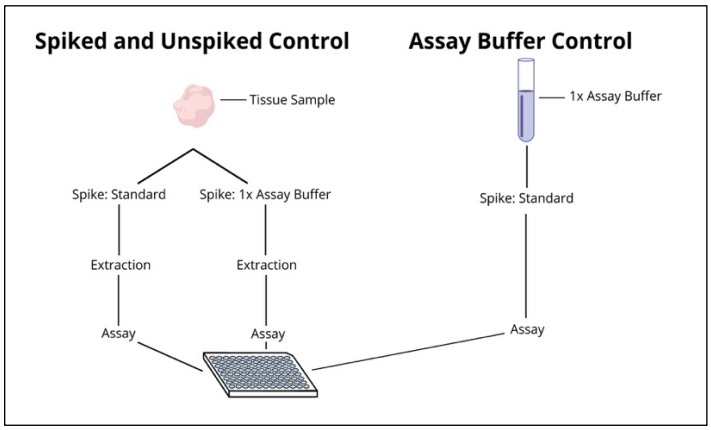

Figure 1. Extraction efficiency workflow diagram for tissue samples.

SAMPLE ANALYTE CONCENTRATION CALCULATION:

For tissue samples, concentrations are often reported as the analyte concentration normalized to tissue mass (e.g. pg/mg tissue). This section outlines the calculation to normalize your sample concentrations to tissue mass using the formula below and provides an example scenario. Concentration units are assay dependent.

$$\textbf{Normalized Analyte Concentration}\left(\frac{\textbf{pg}}{\textbf{mg}}\right) = \frac{\left(\text{Assay Concentration}\left(\frac{\text{pg}}{\text{mL}}\right) \times \text{Assay Dilution Factor} \times \text{Reconstitution Volume (mL)}\right)}{(\text{Tissue Mass (mg)} \times \text{Extraction Efficiency})}$$

Below is a simplified example extraction and the resulting calculations to normalize sample concentration to tissue mass. The volumes described below may need to be adjusted for experimental samples.

1. Weigh 50 mg of tissue in a 50 mL centrifuge tube and add 15 mL of acetonitrile (50 mg Tissue Mass).
2. Homogenize sample, centrifuge at 10,000 x g for 10 minutes, and transfer supernatant to a clean tube.
3. Add 30 mL hexane to the supernatant and incubate for 5 minutes while shaking.
4. After the layers separate, collect the acetonitrile layer into a clean tube and evaporate completely.
5. Reconstitute evaporated sample with 100 µL ethanol and dilute 1:5 with 400 µL 1X Assay Buffer (0.1 mL Reconstitution Volume, 5x Dilution Factor). Use this sample to run the assay following the assay protocol.
6. The assay reported a concentration of 500 pg/mL for this sample. Following the equation above and using a 95% extraction efficiency, the normalized concentration to tissue mass is:

$$\frac{\left(500\frac{\text{pg}}{\text{mL}} \times 5 \text{ Dilution Factor} \times 0.1 \text{ mL}\right)}{(50 \text{ mg} \times 0.95)} = 5.3 \text{ pg analyte/mg tissue}$$