

Steroid solid extraction protocol

Rev 1.0

Organic phase extraction is recommended for extracting steroids from non-liquid matrices, such as dried solids or other organic matter. The following procedure is modified from a standard protocol. Ethanol or ethyl acetate is used because it is a safer alternative to diethyl ether or methylene chloride. Ethanol is also recommended as a means to completely solubilize the dried steroid because certain steroids have limited aqueous solubility. The organic layer is separated and stored.

Required materials

- Steroid standard to allow determination of extraction efficiency
- ACS grade ethanol (or ethyl acetate)
- Glass test tubes

General guidelines

- Ensure that the sample is completely dry before extraction.
- If possible, powder the sample to improve extraction efficiency.
- Remove any large particles, such as grass, if possible.
- Check the extraction efficiency by preparing a steroid solution of known concentration in 1X Assay Buffer. Spike one aliquot of sample with a volume of the steroid solution in 1X Assay Buffer (Control Spike) and one aliquot of sample with the same volume of 1X Assay Buffer only (Control Sample). Extract samples and both controls with Ethanol or Ethyl Acetate as described in "Perform steroid extraction".
- Use a vacuum device (i.e., a SpeedVac™ concentrator) to remove solvents completely and safely.

Perform steroid extraction

1. Weigh out ≥ 0.2 g of dried fecal solid into a tube.
2. Add 1 mL of ethanol (or ethyl acetate) for every 0.1 g of solid sample.
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™ concentrator or under nitrogen.
Note: If only a portion of the organic solvent is being evaporated, ensure final amounts of measured steroid per gram solid accounts for the volume of solution evaporated.
6. Keep dried extracted samples frozen at -20°C or lower in a desiccator.

Dissolve extracted sample

1. Add 100 μL ethanol to extracted sample, followed by at least 400 μL of 1X Assay Buffer.
2. Vortex well and incubate for 5 minutes at room temperature.
3. Vortex and incubate for 5 minutes two more times to ensure complete steroid solubilization.
4. Dilute the solubilized sample $\geq 1:10$ with 1X Assay Buffer, or as directed in the kit manual to ensure ethanol content in the immunoassay wells are $<5\%$.
5. Run reconstituted diluted samples in assay immediately according to insert directions.
6. 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.

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Product label explanation of symbols and warnings

	Catalog Number		Batch code		Temperature limitation		Use by		Manufacturer		Consult instructions for use		Caution, consult accompanying documents
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