Steroid Liquid Sample Extraction Protocol

For our DetectX® Steroid Immunoassay Kits

INTRODUCTION

For an accurate determination of total steroid concentrations in serum, plasma, saliva and other liquid samples, samples may need to be extracted prior to assaying. Extraction eliminates potential interfering substances, such as bulk proteins and lipids. Extraction may also be necessary to concentrate the sample to within the assay's measurement range. If the steroid to be measured and the assay requires serum or plasma samples to be concentrated we would recommend using at least 1-2 mL of serum or plasma.

As with any purification technique, recovery of the desired substance is likely to be incomplete. Therefore, both optimization and quantification of the extraction procedure are recommended for accurate determinations. Either a solid phase or a liquid extraction method may be used. Our preference is the first Liquid Extraction Protocol.

LIQUID EXTRACTION

Materials Needed:

- Steroid standard to allow extraction efficiency to be accurately determined.
- Diethyl Ether or Ethyl Acetate
- Dry ice
- Ethanol
- Speedvac
- Deionized Water

There are two protocols for extraction from a liquid sample. The first protocol allows maximum recovery of steroid but it takes a longer time to complete. The second is better suited for larger number of samples.

First Protocol:

1. Add diethyl ether or ethyl acetate to liquid samples at a 5:1 (v/v) solvent:sample ratio.
2. Mix solutions by vortexing for 2 minutes. Allow solvent layer to separate for 5 minutes.
3. Freeze samples in a dry ice/ethanol bath and pour solvent solution from the top of the sample into a clean tube. Repeat steps 1-3 for maximum extraction efficiency, combining top layer of ether solutions.
4. Dry pooled solvent samples down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept desiccated at -20°C.
5. Redissolve samples at room temperature in the kit-specific Assay Buffer. A minimum of 125µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

Second Protocol:

1. Add five parts of diethyl ether or ethyl acetate to each part of liquid samples.
2. Nutate solutions for 5 minutes or vortex for 2 minutes.
3. Allow phases to separate for 5 minutes.
4. Transfer organic solvent phase to a clean glass test tube containing 1 mL water.
5. Nutate the solvent/water solution for 5 minutes or vortex the mixture for 2 minutes.
6. Allow phases to separate for 2 minutes.
7. Transfer the top organic solvent layer to a clean glass test tube. Steps 1-6 can be repeated for maximum extraction efficiency, combining top layer of solvent solutions.
8. Dry samples down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept desiccated at -20°C.
9. Redissolve samples at room temperature in the kit-specific Assay Buffer. A minimum of 125µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

SOLID PHASE EXTRACTION

Materials Needed:

- Steroid standard to allow extraction efficiency to be accurately determined (see below).
- 200 mg C18 solid phase system columns.
- Vacuum manifold
- Speedvac
- 100% Methanol
- Diethyl Ether
- Deionized Water

Procedure:

1. Condition 200 mg C18 solid phase columns on a vacuum manifold by passing 5-10 mL of 100% methanol through the columns, followed by 5-10 mL of water.
2. Apply liquid samples to individual washed columns.
3. Wash columns with 5-10 mL water. Allow water to drain completely from columns until dry.
4. Elute samples by addition of 2 mL of diethyl ether to the individual columns.
5. Dry samples down in a speedvac for 2-3 hrs. If samples need to be stored they should be kept desiccated at -20°C.
6. Rehydrate samples at room temperature in kit-specific Assay Buffer. A minimum of 125µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

Extraction Efficiency Determination

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 Controls with diethyl ether as described above. Efficiency is calculated as below:

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

Further details on extractions, assays and extraction efficiency can be found at:
www.scribd.com/doc/57985925/18/EXTRACTION-EFFICIENCY