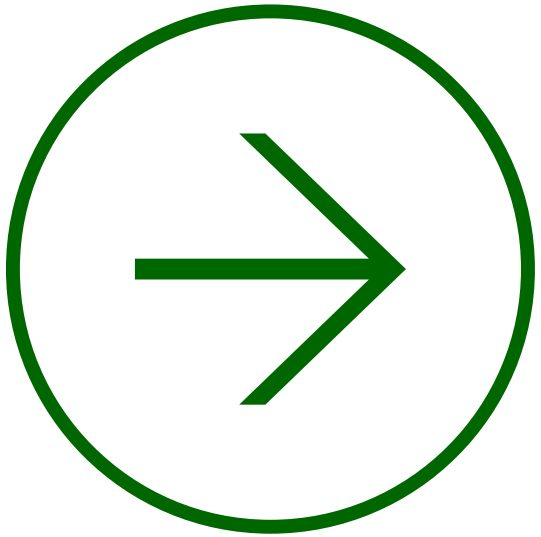
Less is more!

Steroid Liquid Extraction Protocol

For our ZELLX® Steroid Immunoassay Kits



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INTRODUCTION

Efficient extraction of samples might be a necessity for an accurate determination of total steroid concentrations in serum, plasma, saliva and other liquid samples. Extraction may also be necessary to concentrate the sample to within the assay's measurement range. Extraction eliminates potential interfering substances, such as bulk proteins and lipids. We would recommend using at least 1-2 mL of serum or plasma for the steroid measurement If the assay requires serum or plasma samples to be concentrated. 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. We recommend the first Liquid Extraction Protocol.

MATERIALS NEEDED (for Liquid extraction)

- Steroid standard for extraction efficiency determination
- ACS Grade Ethanol (or Ethyl Acetate)
- Dry Ice, Ethanol, Deionized water
- Speedvac

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There are two protocols for extraction from a liquid sample. The 1st protocol allows maximum recovery of steroid, however, it takes a longer time to complete. The 2nd is better suited for larger number of samples.

PROCEDURE 1

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 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement

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PROCEDURE 2

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 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

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MATERIALS NEEDED (for Solid Phase Extraction)

- Steroid standard for extraction efficiency determination
- Diethyl Ether, Pure Methanol, Deionized water
- Speedvac, Vacuum manifold
- 200 mg C18 solid phase system columns

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 125 µL of the Assay Buffer is recommended for reconstitution to allow for duplicate sample measurement.

EXTRACTION EFFICIENCY

Prepare 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 earlier.

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)

Technical Support: Customer satisfaction is very important to us. Please call us at +49(0)30 81309085 or send an email to technical[at]zellx.de with your technical questions. A member of our scientific staff will be glad to assist you.
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