DetectX®

Peptide/Protein Extraction Protocol

For our DetectX® Immunoassay Kits

ARBOR ASSAYS®

INTRODUCTION

If your samples are serum or plasma, you may need to extract the antigen from the contaminating peptides and proteins. We have provided an excess amount of antigen standard so that you may use to determine if extraction is required. For example, if you are working with serum, you may spike it with known amounts of standard and check if they are accurately determined by the assay with and without extraction. Extraction eliminates potentially interfering substances, such as bulk proteins and lipids. Extraction may also be necessary to concentrate the sample to within the measuring range. 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.

Sample Collection

Collect plasma samples into EDTA collection tubes. Centrifuge blood at 1,600 x g for 15 minutes at 4°C. Collect the top (plasma) layer. As soon as possible after collection add a Protease Inhibitor Cocktail, such as Sigma P1860-1ML, 0.5 µL/mL of plasma. Either use the sample immediately or freeze in aliquots at -80°C or below.

C18 Sep-Column Extraction Method

The following generic protocol is meant to help users with little experience in extracting their samples. It should be applicable to different biological fluids but should not be thought of as an optimized protocol for any particular antigen.

MATERIALS NEEDED

- C18 Sep-Pak Column containing >50 mg of C18
- Binding Buffer (BB) - 1% trifluoroacetic acid (TFA, HPLC Grade).
- Elution Buffer (EB) - 60% acetonitrile (HPLC Grade), 1% TFA, and 39% distilled water.

EXTRACTION PROCEDURE

1. Mix an equal amount of Binding Buffer (BB) to the plasma and vortex.
2. Centrifuge at 6,000 x g to 17,000 x g for 20 minutes at 4°C. Collect supernatant.
3. Equilibrate a C18 column by washing with 1 ml Elution Buffer (EB) followed by 3 X 3 ml BB.
4. Load the plasma/BB solution from step b) onto the washed C18 column.
5. Slowly wash the column with BB (3 ml, twice) and discard the wash. A light vacuum (10 sec/drop) may be applied to the column.
6. Elute the peptide slowly with Elution Buffer (EB) (3 ml, once) and collect eluant in a polypropylene tube. A light vacuum may be applied as in previous step.
7. Remove acetonitrile solvent in a centrifugal concentrator. Freeze-dry resulting water/TFA solution to dryness.
8. Dissolve the residue in a suitable volume of Assay Buffer provided with the kit.

TISSUE AND CSF

Add 5mL of Binding Buffer for every gram of -80°C frozen tissue. Homogenize using a ultrasonic or Parr type homogenizer. Follow steps 2-8 above. For CSF samples, add 1 mL BB to every mL of CSF. Follow steps 2-8 above.