Peptide/protein extraction protocol

Rev 1.0

When starting with serum or plasma samples, your antigen of interest may need to be extracted from contaminating peptides and proteins. To determine if extraction is required, spike the samples with a known amount of standard and check if they are accurately measured by the assay with and without extraction. An excess amount of antigen standard is provided to perform this procedure.

Extraction eliminates potentially interfering substances, such as bulk proteins and lipids. It can also be used to concentrate the sample so it falls 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.

Required materials

- C18 Sep-Pak™ Column containing >50 mg of C18
- Binding Buffer (1% trifluoroacetic acid (TFA, HPLC grade))
- Elution Buffer (60% acetonitrile (HPLC grade), 1% TFA, and 39% distilled water)
- Protease inhibitor cocktail (e.g., Sigma P1860-1ML)

Sample preparaton guidelines

1. Collect plasma samples into EDTA collection tubes.
2. Centrifuge blood at 1,600 × *g* for 15 minutes at 4°C. Collect the top layer (plasma).
3. Add 0.5 µL of protease inhibitor cocktail for every 1 mL of plasma as soon as possible after collection.
4. Use the sample immediately or freeze in aliquots at –80°C or lower.

Perform sample extraction (serum or plasma)

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

1. Mix an equal amount of Binding Buffer to the plasma and vortex.
   - For **plasma** samples, mix an equal amount of Binding Buffer to the plasma and vortex.
   - For **tissue** samples, add 5mL of Binding Buffer for every gram of –80°C frozen tissue. Homogenize samples using a ultrasonic or Parr-type homogenizer.
   - For **CSF** samples, add add 1 mL of Binding Buffer for every 1 mL of CSF.
2. Centrifuge at 6,000– 17,000 × *g* for 20 minutes at 4°C. Collect supernatant.
3. Equilibrate a C18 column by washing with 1 mL Elution Buffer.
4. Wash the C18 column with 3 mL of Binding Buffer three times.
5. Load the plasma/ Binding Buffer solution from step 2) onto the washed C18 column.
6. Apply light vacuum to the column (10 seconds/drop) and slowly wash the column with 3 mL of Binding Buffer twice. Discard the wash.
7. Apply light vacuum to the column (10 seconds/drop) and elute the peptide slowly with 3 mL of Elution Buffer. Collect eluate in a polypropylene tube.
8. Remove acetonitrile solvent in a centrifugal concentrator. Freeze-dry the resulting water/TFA solution to dryness.
9. Dissolve the residue in a suitable volume of 1X Assay Buffer provided with the kit.

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