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S-5000
Extraction kit

Contents:

Buffer A (x 2) - 1% trifluoroacetic acid (Y-1040)
Buffer B (x 1) - 60% acetonitrile with 1% trifluoroacetic acid (Y-1045)
Sep-Column (50 columns) - C18 sorbent (Y-1000)
The kit components should be enough to extract 50 samples.

Storage:

Kit components can be stored at room temperature. The Sep-columns can be kept in its original package and must be stored in desiccators.

Recommended Use:

Extraction of Peptide from Plasma

1. Add an equal amount of Buffer A to the plasma. For example, if you are using 1 ml of plasma, acidify with 1 ml of Buffer A. Centrifuge at 6,000xg to 17,000xg for 20 minutes at 4°C. Discard any pellet that may be present.
2. Equilibrate a SEP-COLUMN containing 200 mg of C18 (Cat No. Y-1000) by washing with Buffer B or Buffer D (100% Acetonitrile) (1 ml, once) followed by Buffer A (3 ml, three times).
3. Load the plasma solution onto the pre-treated C-18 SEP-COLUMN.

Note: For steps 4 and 5, a light vacuum (10 sec/drop) may be applied to the column.

4. Slowly wash the column with Buffer A (3 ml, twice) and discard the wash.
5. Elute the peptide slowly with Buffer B (3 ml, once) and collect eluant in a polypropylene tube.
6. Evaporate eluant to dryness using a centrifugal concentrator or by a suitable method;

   Ex: Lyophilizer. Recommended to freeze eluant with dry ice/methanol for fastest freeze.
7. Dissolve the residue in a suitable volume of assay (EIA or RIA) buffer such that the concentration of the substance of interest will fall close to the IC_{50} (within the measuring range).
8. The total time for extraction should be 0.5 days for extraction and 0.5 days for lyophilization. Once the sample is lyophilized, it can be stored at -70°C before assaying, but should be assayed as soon as possible.

NOTE: This is a generic extraction protocol, which can be used for multiple types of samples. If a more suitable extraction method is found in literature, we encourage the researcher to use it.