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Cat. No. Y-1040
Buffer A
(Extraction Wash buffer)

Reconstitution Information:

This buffer is ready-to-use as a wash buffer in a solid phase extraction protocol using a C-18 Column (Cat # Y-1000). Each 500 ml solution contains 1% trifluoroacetic acid in USP water.

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. Elude 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 RIA buffer for radioimmunoassay as follows: For a normal subject, dissolve in 250 µl RIA Buffer for a two-tube assay. Aliquot 100 µL into each tube (50 µl is left over). If each tube is found to contain 3.963 pg/tube, then the total level of peptide in plasma sample = 3.962 pg/tube x 2.5 tube = 9.9 pg. If upon assaying, the peptide value exceeds or does not fall in the range of detection, dilute or concentrate the samples accordingly.

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.