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Design and preparation of synthetic reference peptides for APP/Aβ TOMAHAQ proteomics V.2

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TOMAHAQ-based targeted proteomics relies on heavy-labeled reference peptides for multi-plexed quantification of peptides of interest within a set of samples. The APP amyloid precursor protein is thought to be proteolytically processed within the endolysosomal system by β-secretase and ϒ-secretase to yield various forms of Aβ. To quantitatively track APP products, we describe a protocol for design and generation of synthetic reference peptides for TOMAHAQ analysis. We also include reference peptides for the extracellular and cytosolic domains.

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1 Design APP peptides corresponding to extracellular and cytosolic regions based on data available in Peptide Atlas which are predicted to have favorable LC-MS properties. See attached Table.

2 Design half-tryptic peptides based on the cleavage sites for BACE1 and ϒ-secretase. See attached table.

3 Order commercial synthesis of peptides from Biomatik and Thermo Fischer Scientific, or similar.

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4 Reconstitute the peptides with 3% acetonitrile/0.1% formic acid, and quantify the concentration using Pierce Quantitative Fluorometric Peptide Assay.

5 Mix 10 µL of 200 µM peptide in 200 mM EPPS buffer (pH 8.5) with 1 µL of 5 µg/µL super-heavy TMT reagent (TMTsh). Incubate at 25 °C for 1 hr then check the labeling efficiency by LC-MS.

6 Add extra TMTsh to under-labeled peptides until > 95% labeling on both N-terminus and lysine residues is reached.

7 Desalt the reaction mixture using C18 StageTip.

8 Resuspend the peptides in 5% acetonitrile/8% formic acid, and pool the peptides.

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