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diDO-IPTL protocol

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Isobaric peptide termini labeling (IPTL) is a quantification method which permits relative quantification using quantification points distributed throughout the whole tandem mass spectrometry (MS/MS) spectrum. It is based on the complementary derivatization of peptide termini with different isotopes resulting in isobaric peptides.

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DAY 1

1 Prepare dried MS-grade trypsin, 2ug per sample to be labeled. Note that separate aliquots of dried trypsin must be used for ^{16}O- and ^{18}O-labeling.

2 Prepare two separate N-methylmorpholine (NMM) and acetic acid O-isotope exchange buffers with ^{16}O and ^{18}O (>98%) enriched water. Volume ratio of buffer components is 48.5 H_2O : 1 NMM : 0.5 acetic acid (pH 7.4). The total volume of buffer to prepare depends on how many

peptide samples are to be labeled. The protocol below is for 40µL of O-isotope exchange buffer per sample (~10-20 µg of peptide). For samples with small amounts of peptide, the concentration can be raised by adding only 20µL buffer per sample and halving the volumes of all subsequent reagent additions.

3 Redissolve dried trypsin aliquots in their respective O-isotope exchange buffers and transfer 40µl to each tube of dried peptide sample. KEEP TRACK of which tubes received which buffer.

4 Parafilm the tops of the tubes and incubate at 37ºC overnight.

DAY 2

5 After overnight incubation, add 2µl of 11.3M monochloroacetic acid (prepared in LC-MS grade water) to each peptide tube to lower pH down to 2.6.

6 Prepare 16% CH_2O and CD_2O formaldehyde solutions, at least 2µl per sample, diluting stocks with LC-MS grade water if necessary.

7 Prepare 4.8M $NaBH_3CN$ in LC-MS water. Tare 1.5mL tube, add a small amount of $NaBH_3CN$ powder in fume hood, weigh, dissolve.

8 Add 2µL of CH_2O to each ^{18}O tube and 2µL of CD_2O to each ^{16}O tube.

9 Add 2µL 4.8M $NaBH_3CN$ to each tube.

10 Mix well and incubate at 45ºC for 1 hr. While waiting, prepare 5M ammonium formate in LC-MS water.

11 Add 2µL 5M ammonium formate to each tube and mix well.

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12 Add 8µL of formic acid to each tube and mix well. Total sample volume is ~56µl. ^{16}O- and ^{18}O-labeled samples are ready to be mixed and analyzed by LC-MS.

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