AUG 08, 2023

TMT labelling

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ABSTRACT

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Protocol Citation: Louise Uoselis 2023. TMT labelling. **protocols.io** https://protocols.io/view/tmt-labelling-cyesxtee

Protocol status: Working
We use this protocol and it's working

Created: Aug 08, 2023

Last Modified: Aug 09, 2023

PROTOCOL integer ID: 86194

Keywords: ASAPCRN

1 Reconstitute lyophilised peptides in 25 µL of 100 mM TEAB by vortexing each sample for ~ 00:00:10, and then leaving to sit at Room temperature for 00:15:00. Each sample was then sonicated for 00:05:00 in a waterbath sonicator in an ice slurry. 20m 10s

2 Determine the peptide concentration of each sample spectroscopically

3 Aliquot 10 µg of peptides for each sample into a LoBind microfuge tube and add 100 mM triethylammonium bicarbonate to a final volume of 100 uL for each sample.

4 For the pooled batch control, divide 100 µg by the total number of samples, and aliquot that amount of peptides from each sample into one tube. Make up the pooled control volume to 100 uL with 100 mM triethylammonium bicarbonate (can lyophilise the samples to reduce the volume if the total pooled volume exceeds 100 uL).

5 Bring the TMT labels to Room temperature.

6 Reconstitute each label in acetonitrile as per the manufacturer's instructions.

7 Add the required volume of the designated label to each sample, and vortex each sample for ~ 00:00:05 to mix. 5s

8 Leave samples to incubate at Room temperature for 01:00:00 (static) 1h

9 Add hydroxylamine to a final concentration of 26%/sample, vortex each sample for ~ 00:00:05, and incubate at Room temperature for 00:15:00 to quench the TMT labelling reaction 15m 5s

10 Combine the samples into their designated batches, and lyophilise the pooled samples. Seal each tube with parafilm and store at -80 °C for downstream processing.