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## TMT labelling

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ABSTRACT

TMT labelling

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<https://protocols.io/view/tmt-labelling-cyesxtee>

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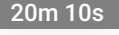
















**Protocol status:** Working  
We use this protocol and it's working


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86194

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- 1 Reconstitute lyophilised peptides in  25 µL of 100 mM TEAB by vortexing each sample for  20m 10s  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.
- 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 µL 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 µL with 100 mM triethylammonium bicarbonate (can lyophilise the samples to reduce the volume if the total pooled volume exceeds 100 µL).
- 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 ~  5s  00:00:05 to mix.
- 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 ~  15m 5s  00:00:05, and incubate at  Room temperature for  00:15:00 to quench the TMT labelling reaction

- 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.