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Stable isotope dimethyl labeling

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[The protocol is designed to label 25-30 µg tryptic peptides]

[All preparation should be fresh]

1. Extract proteins from cell lines or biological tissue using 8M urea in 100 mM TEAB, protease inhibitor cocktail.
2. Quantify proteins using BCA method.
3. Start with **25- 30 µg** protein extract, proceed with amine- free, In- solution trypsin digestion protocol as follows;

#scale up or down based on volume (optimally 30-50 µl)

a) **30 µl** 8 M Urea buffer [240 mg urea, 100 µl of 100 mM TEAB, 220 µl MilliQ, protease inhibitors]

b) **0.2µl** 1M TCEP (tris (2-carboxyethyl) phosphine) in milliQ
mol. Mass 250.19 g mol^{-1}

c) Incubate at room temperature for 30 minutes

d) **0.5 µl** of 500 mM IAA in milliQ

e) Incubate at room temperature for 30 min in dark

f) **90 µl** 100 mM TEAB to get final urea dilution around 2M

g) Trypsin (1:20): prepare 0.5ug/µl trypsin solution and add 2 µl

h) Add **4µl** 100 mM CaCl2

i) Incubate overnight at 37^{o}C in shaker (600 rpm)

j) Spin down for 30 min at max speed at room temperature

 # Don't acidify the sample

4. Start with trypsinized peptides in TEAB and urea buffer without final acidification.

[above; total 125 µl contains 30 µg with a final conc 0.24 ug/µl]

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5. Take **100 µl** [contains 24 µg digested peptide] for subsequent labeling, make sure pH 5.0~8.0. (check with pH paper)

6. Add **4µl** of 4% (vol/vol) of either formaldehyde (CH_2O), Deuterated formaldehyde (CD_2O), or C13, deuterated formaldehyde ($^{13}CD_2O$) to be labeled with light, intermediate, or heavy dimethyl, respectively. (CH_2O is diluted **1:8** (2µl F+ 16 µl milliQ), CD_2O and $^{13}CD_2O$ are diluted 1:4 (2µl F+ 8 µl milliQ))
7. Mix briefly, spin down the sample

8. Prepare cyanoborohydride, cyanoborodeuterate solutions to 0.6M ($NaBH_3CN$, and $NaBD_3CN$) (prepare fresh). [0.018 g/500 µl milliQ]

9. Add **4 µl** of 0.6M $NaBH_3CN$ to light and intermediate labeling, and **4 ml** of 0.6 M $NaBD_3CN$ heavy labels

Light	Medium	Heavy
+28.0313	+32.0564	36.0757
CH_2O	CD_2O	$_{13}CD_2O$
$NaBH_3CN$	$NaBH_3CN$	$NaBD_3CN$

10. Incubate at RT for 1 hour, (preferably in the hood in a shaker)

11. Quench reaction (in the hood) by the addition of **15µl** of 200mM ammonium bicarbonate (or 1% ammonia solution)-mix [7.9 g/500 µl]
12. Add 8µl of formic acid to further quench reaction and acidify sample.
13. Stage tip the sample (30 ug/130 µl with conc 0.2 ug/µl)