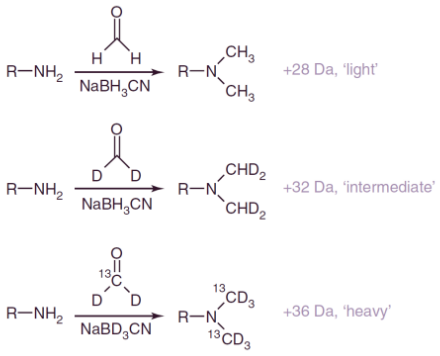
PROTEOMICS CORE FACILITY COLOGNE

Dimethyl labeling on StageTips

This protocol combines stable isotope chemical labeling of peptides and purification of peptides using StageTips. C18 StageTip purification removes salts, but cannot get rid of detergents! If your sample contains compounds such as Glycerol, PEG, Triton, Tween, SDS, NP40 etc. contact the Core Facility before using this protocol!

Reagents & Solutions

- Buffer A: 0.1% formic acid in water *
- Buffer B: 0.1% formic acid in 80% Acetonitrile *
- Formic acid, 10% in water *
- 100% Methanol *
- Sodiumcyanoborohydride ($NaBH_3CN$) *
- Sodiumcyanoborodeuteride ($NaBD_3CN$) *
- Formaldehyde, 37% *
- D-Formaldehyde, 20% *
- D-^{13}C-Formaldehyde, 20% *
- P1: 100 mM NaH_2PO_4 (6,9 g in 500 mL water) *
- P2: 100 mM Na_2HPO_4 (8,9 g in 500 mL water) *



Equipment

- C18 StageTips, packed in 200 µL pipette tips *
- 2 mL Eppendorf collection tubes *
- Centrifugal adaptors for StageTips *
- Eppendorf benchtop centrifuge *

* available in the core facility

Protocol

1. Prepare labeling reagent stock buffers[1]:
    a. Phosphate buffer: 1.13 mL of **P1** + 3.87 mL of **P2** + 5.0 mL water
    b. A1: 600 mM $NaBH_3CN$ (18.9 mg in 0.5 ml Water, in 2 mL Eppendorf tube)[2]
    c. A2: 600 mM $NaBD_3CN$ (19.8 mg in 0.5 ml Water, in 2 mL Eppendorf tube)[2]
    d. F1: 4% Formaldehyde (10.9 µl 37% + 89.1 µl water)[3]
    e. F2: 4% D-Formaldehyde (20 µl 20% + 80 µl water)[3]
    f. F3: 4% D-13C-Formaldehyde (20 µl 20% + 80 µl water)[3]

2. Prepare final labeling reagent solvents[4]:
    a. LIGHT:
        - 1.35 mL of Phosphate buffer
        - 75 µL of **A1**
        - 75 µL of **F1**

    b. MEDIUM:
        - 1.35 mL of Phosphate buffer
        - 75 µL of **A1**
        - 75 µL of **F2**

    c. HEAVY:
        - 1.35 mL of Phosphate buffer
        - 75 µL of **A2**
        - 75 µL of **F3**

3. Equilibrate the StageTips, use one StageTip for each sample to be labelled[5]:
    a. add 20 µL Methanol to the StageTip → centrifugation at 2.600 rpm for 2 min
    b. add 20 µL buffer B to the StageTip → centrifugation at 2.600 rpm for 2 min
    c. add 20 µL buffer A to the StageTip → centrifugation at 2.600 rpm for 2 min
    d. add 20 µL buffer A to the StageTip → centrifugation at 2.600 rpm for 1 min
    e. make sure to keep a little bit (approximately 1 mm)[6] of buffer A on top of the C18 material. If too much buffer A remains on top centrifuge ONLY THESE StageTips a little bit longer

4. Discard the solvents from the collection tubes before you continue

5. Sample labeling and purification:
    a. Load the samples onto the StageTips
    b. Centrifugation at 2.600 rpm for 4 min, discard flowthrough
    c. Add 30 µL buffer A onto the StageTips
    d. Centrifugation at 2.600 rpm for 3 min, discard flowthrough
    e. Load **50 µL** of the respective LIGHT/MEDIUM/HEAVY labeling reagent on the StageTip
    f. Centrifugation at 1.000 rpm for 10 min, discard flowthrough[7]
    g. Repeat steps e) and f) two more times[8]

h. Add 30 µL buffer A onto the StageTips
i. Centrifugation at 2.600 rpm for 3 min, discard flowthrough
j. Dry the StageTip with a syringe & store them at 4°C

Notes

[1] Always prepare these buffers fresh on the day of use. Use Parafilm to seal the chemical containers after taking out the necessary amounts.

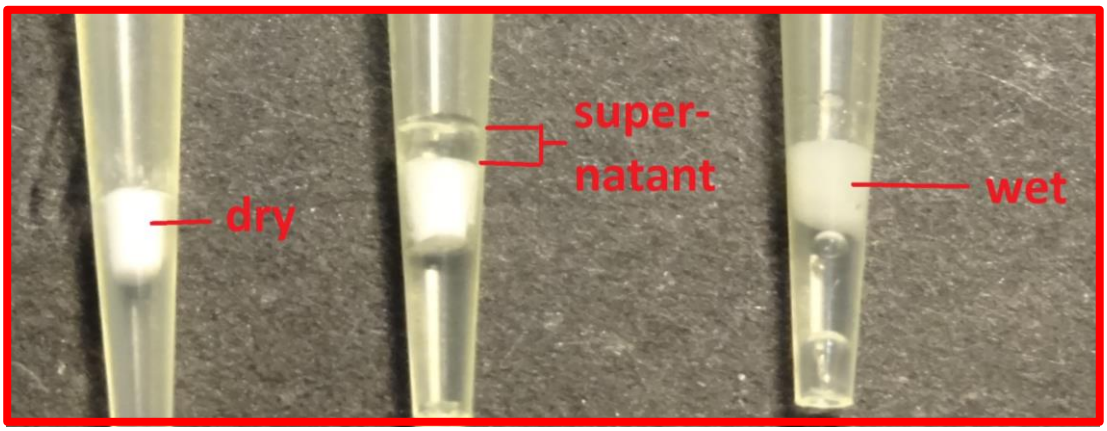
[2] Never use the same spatula for $NaBH_3CN$ and $NaBD_3CN$. We have dedicated spatulas for both chemicals. IMPORTANT: Both compounds are very toxic. To prepare the solvents weigh in an empty Eppendorf tube ("zero" the balance). Under the fume hood add a tiny amount of $NaBH_3CN$ or $NaBD_3CN$ to the tube. Close the lid and weigh the tube. Adjust the amount of water to dissolve the compound under the fume hood to achieve the correct concentration.

[3] Formaldehyde is very toxic. Prepare the diluted solvents under a fume hood. First pipet water into the tubes, then add the formaldehyde under the fume hood.

[4] Store at 4°C until use, but no longer than 24h. 2 mL of labeling reagent is sufficient to label 13 samples.

[5] Centrifugation times can vary, depending on sample volume, C18 phase tightness and sample composition. Adjust if necessary.

[6] IMPORTANT: The Stage tips should not be completely dry after this step! See also example picture:



[7] IMPORTANT: The labeling solution should not pass through the StageTip in less than 10 min. Adjust the centrifugation speed if necessary!

[8] Before you proceed to the next step make sure that the labeling solutions have almost completely passed through the StageTips.