Methylated peptides purification by strong cation exchange

Boisvert Lab, december 2023, Adapted from Wang *et. al*., Analytical Chemistry, 2016, 88, 11319-11327.

Protein digestion

1. Solubilize proteins directly in a solution of 8 M urea, 1 M NH_4HCO_3, 20 mM HEPES pH 8.0 (lysis buffer) (in MS-grade water). For a total cell extract, resuspend directly the cell pellet coming from a 100mm petri dish of confluent cells (~2 mg of total protein) in 500 µl of lysis buffer. Sonicate on ice (12 cycles of 5 sec PµLSE /5 sec OFF, intensity of 20-25%), centrifuge 16,000 x g for 10 min at 4ºC and transfer the supernatant to a new lobind tube. Quantify your cell extract using BCA kit (compatible with 3M urea, so do not forget to dilute your sample). Generaly speaking, transfer 250 µl of cell extract containing up to 1 mg of total proteins to a new lobind tube and proceed to the next step.

2. Add DTT (5 mM final concentration). Boil at 95ºC for 2 minutes, then incubate at RT for 30 min.

3. Add chloroacetamide (7.5 mM final concentration). Incubate at RT for 20 min in the dark.

4. Add 750 µl of 1M NH_4HCO_3 (in MS-grade water) to the mixture so that the final concentration of urea is reduced to 2 M.

5. Add 10 µg trypsin and incubate at 30ºC overnight.

6. Acidify the sample to a final concentration of 0.2% TFA.

HYPERSEP cleaning

We use HYPERSEP C18, 100 mg /1 ml column (Thermo Scientific, cat: 60108-302). NB: you can increase the flow of the liquid by pushing air slowly with your P1000 pipet and a filter-tip.

1. Load 3 times 1 ml of 100% acetonitrile (wetting).

2. Load 3 times 1 ml of 0.1% TFA (equilibration).

3. Load your sample in the column with a flow of 0.5-1 ml/min, collect and reload the effluent twice.

4. Load 3 times 1 ml of 0.1% TFA (wash).

5. Elute 3 times with 500 µl of elution buffer (50% acetonitrile /1% formic acid) with a flow of 0.5-1 ml/min, collect the eluate in a new lobind tube.

6. Evaporate your sample using speedVac.

Methylated peptides enrichment

We use Pierce Strong Cation exchange spin columns, mini (cat: 90008).

Materials required

• Collection tubes: lobind microcentrifuge tubes, 2 ml.
• pH paper.
• BRUB (5 mM phosphoric acid, 5 mM boric acid, 5 mM acetic acid in MS-grade water).
• BRUB (pH 12.0), adjust the pH to 12.0 with NaOH 1M.
• Loading buffer (60% acetonitrile/40% BRUB, pH 2.5), adjust the pH to 2.5 with HCl 1M.
• Wash buffer (80% acetonitrile,/20% BRUB, pH 9.0), adjust the pH to 9.0 with NaOH 1M.
• Elution buffer 1 (60% acetonitrile/40% BRUB, pH 9.0), adjust the pH to 9.0 with NaOH 1M.
• Elution buffer 2 (60% acetonitrile/40% BRUB, pH 10.0), adjust the pH to 10.0 with NaOH 1M.
• Elution buffer 3 (60% acetonitrile,/40% BRUB, pH 11.0), adjust the pH to 11.0 with NaOH 1M.
• Elution buffer 4 (30% acetonitrile/70% BRUB, pH 12.0), adjust the pH to 12.0 with NaOH 1M.
• Elution buffer 5 (100% BRUB/1M NaCl, pH 12.0), adjust the pH to 12.0 with NaOH 1M.

Suspend peptide sample

1. Completely resuspend your peptides (1 mg) in 400 µl of Loading buffer. Use vortex mixer with tube stand if necessary. **Note**: For optimal resµlts, lyophilized peptide samples must be entirely dissolved in Loading buffer.
2. Optional: Verify pH of resuspended sample is <3 using pH paper.

Prepare column

1. Insert a SCX column into a 2 ml microtube, add 400 µl of BRUB (pH 12.0) to the column and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through. Repeat once.
2. Equilibrate the SCX column by adding 400 µl of Loading buffer to the column and centrifuge at 2,000 × g for 5 minutes. Discard the flow-through. Repeat once.

Bind methylated peptides

1. Transfer the equilibrated SCX column into a new 2 ml microcentrifuge tube.

2. Add 400 µl of suspended peptide sample to the SCX column. Centrifuge at 2000 × g for 5 minutes.
3. Reapply sample in the microcentrifuge tube to the SCX column. Centrifuge at 2000 × g for 5 minutes. If desired, retain the flow-through for analysis.

Wash column

1. Transfer the SCX column into a new 2 ml microcentrifuge tube.
2. Add 400 µl of Wash buffer to the SCX column. Centrifuge at 2000 × g for 5 minutes. Discard the flow-through (or if desired, retain the flow-through for analysis). Repeat twice (for a total of 3).

Elute column

1. Transfer the SCX column into a new 2 ml microcentrifuge tube.
2. Add 400 µl of Elution buffer 1 to the SCX column. Centrifuge at 2000 × g for 5 minutes. Keep the flow-through (this will be Elution 1).
3. Transfer the SCX column into a new 2 ml microcentrifuge tube.
4. Add 400 µl of Elution buffer 2 to the SCX column. Centrifuge at 2000 × g for 5 minutes. Keep the flow-through (this will be Elution 2).
5. Transfer the SCX column into a new 2 ml microcentrifuge tube.
6. Add 400 µl of Elution buffer 3 to the SCX column. Centrifuge at 2000 × g for 5 minutes. Keep the flow-through (this will be Elution 3).
7. Transfer the SCX column into a new 2 ml microcentrifuge tube.
8. Add 400 µl of Elution buffer 4 to the SCX column. Centrifuge at 2000 × g for 5 minutes. Keep the flow-through (this will be Elution 4).
9. Transfer the SCX column into a new 2 ml microcentrifuge tube.
10. Add 400 µl of Elution buffer 5 to the SCX column. Centrifuge at 2000 × g for 5 minutes. Keep the flow-through (this will be Elution 5).
11. Speedvac to dryness.
15. Resuspend in 300 µl 0.1% TFA and desalt using C18 tips.

Notes

1. Remember to wear gloves at all times during the procedure. Don't scratch your head, armpits, genital, or any part of your body during the procedure. Keratin contamination is less an issue after peptide digestion, as whole keratin proteins will not interfere with MS analysis.
2. When you make any stock solutions, avoid using any spatµla that have not been cleaned thoroughly by water and ethanol. In fact, we routinely forgot using spatµla at all, and tip the solid chemical into clean eppendorf tubes.
3. Peptides tend to stick to plastic surfaces on storage. We use 1.5 ml lobind tubes that are designed for storing peptides at low concentration.