In-solution Digestion of Proteins with deoxycholic acid

Boisvert Lab, January 2024

It is difficult to solubilize SDC at a solution of 10%. Incubate at 50°C with shaking until complete solubilization. Do not keep your solution at 4°C because it will precipitate. In that case, simply incubate again your solution at 50°C with shaking until complete solubilization. Avoid incubation on ice during all the procedure (even during sonication).

1. Solubilize proteins directly in a solution (made in MS-grade water) of 50 mM NH_4HCO_3, 20 mM HEPES pH 8.0 (lysis buffer without SDC). How much sample to use depends on the amount of proteins, and the purpose of your experiment. 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 125 µl of lysis buffer without SDC. Add 125 µl of 10% sodium deoxycholate (SDC), 50 mM NH_4HCO_3, 20 mM HEPES pH 8.0 (lysis buffer with SDC), so the final concentration of SDC is 5%. Heat the sample at 80°C for 10 minutes. Sonicate (12 cycles of 5 sec PULSE /5 sec OFF, intensity of 20-25%), heat the sonicated sample again at 80°C for 10 minutes, centrifuge at 16,000 x g for 10 min at room temperature and transfer the supernatant to a new lobind tube. Quantify your cell extract using BCA kit (compatible with 5% SDC). Generaly speaking, transfer 25 µl of cell extract containing up to 50 µg of total proteins to a new lobind tube and proceed to the next step.

2. Add DTT (10 mM final concentration). Heat at 60°C for 20 minutes.

3. Add chloroacetamide (20 mM final concentration). Incubate at RT for 30 min in the dark.

4. Add 225 µl of 50 mM NH_4HCO_3 (in MS-grade water) to the mixture so that the final concentration of SDC is reduced to 0.5%.

5. Add 1µg trypsin and incubate at 37°C overnight.

6. Acidify the sample to a final concentration of 0.5 % TFA and vortex for 2 minutes. If there is no white precipitate appearing, add an extra TFA to reach a final concentration of 1% TFA and vortex again for 2 minutes.

7. Centrifuge at 16,000 x g for 10 min. The precipitate is the SDC. Transfer the supernatant to a new lobind tube.

(8). (optional) Add 250 µl of 100% ethyl acetate containing 0.5% TFA and vortex for 2 minutes. Centrifuge at 16,000 x g for 10 min to obtain aqueous and organic phases. The organic phase (upper phase containing traces of SDC) is carefully removed, the remaining aqueous phase is collected, mixed with the precedent supernatant. The tube is left open under the hood for 10 minutes in order to remove traces of ethyl acetate. Although not essential, that optional step 8 helps to remove traces of SDC, thus potentially helping the ionization part during MS processing of the sample.

8. (or (9)) Desalt using a ZipTip.

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 spatula that have not been cleaned thoroughly by water and ethanol. In fact, we routinely forgot using spatula 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.

Adapted from:
Ileana R. León, Veit Schwämmle, Ole N. Jensen, Richard R. Sprenger. Quantitative Assessment of In-solution Digestion Efficiency Identifies Optimal Protocols for Unbiased Protein Analysis, Molecular & Cellular Proteomics,Volume 12, Issue 10, 2013, Pages 2992-3005.