On-Beads Trypsin Digestion

Boisvert Lab, December 2020

This method allows better recovery of the immunoprecipitated proteins, at the price of increased contamination with antibody and increased complexity of the sample. **Digestion including reduction and alkylation:** this step is required to prevent disulfide bonds between peptides, thus increasing peptide coverage. **You can use this protocol for streptavidin pull-down (e.g. BioID system), GST pull-down, His pull-down, MBP pull-down and all nano-TRAP beads from Chromotek (e.g. GFP-TRAP).**

Digestion without reduction and alkylation: there are several reports indicating that antibodies **(e.g. IgG)** which have not been reduced may be partially resistant to trypsin, which could help reduce the amount of antibodies in the sample.

Always use MS-grade water for all of your solutions, as well as lobind tubes for your samples.

Digestion including reduction and alkylation

1) Wash beads 5 times with 20 mM ammonium bicarbonate (NH_4HCO_3). Remove as much wash buffer as possible, beads can be kept at -20°C until use.

2) Add an equal bead volume of 10 mM DTT in 20 mM NH_4HCO_3 to the beads for complete immersion (usually 50 µl is fine), mix and incubate at 60ºC for 30 minutes with shaking at 1250 rpm.

3) After a quick spin and cooling, add an equal volume of 15 mM chloroacetamide (ClAA) in 20 mM NH_4HCO_3 (usually 50 µl is fine) to the DTT/bead suspension, mix and incubate in the dark for 1 hour with shaking at 1250 rpm.

4) Add 1M DTT to increase the concentration to 15 mM (usually add 1 µl of 1M DTT to the 100 ul (total) is fine) to quench the ClAA and wait 10 minutes with shaking at 1250 rpm.

5) Add 1 µg of trypsin to the beads.

6) Incubate at 37°C overnight with shaking at 1250 rpm.

7) Stop trypsin digestion by acidifying to a final concentration of 1% formic acid (FA) and centrifuge at 2,000 x g for 3 min.

8) Transfer the supernatant to a new 1.5 ml protein lobind tube.

9) Resuspend beads in 100 µl of a 60% acetonitrile (CH_3CN)/ 1% FA solution, incubate at room temperature for 5 min with shaking at 1250 rpm.

10) Centrifuge at 2,000 x g for 3 min and transfer this second supernatant with the first supernatant.

11) Dry samples in the speed vac.

12) Resuspend in 300 µl of 0.1% trifluoroacetic acid (TFA) to desalt on ZipTip (100 µl size).

Digestion without reduction and alkylation (reduction and alkylation are performed after digestion)

1) Wash beads 5 times with 20 mM NH_4HCO_3. Remove as much wash buffer as possible, beads can be kept at -20°C until use.

2) Add 2 bead volumes of diluted trypsin (10 ng/µl in 20 mM NH_4HCO_3) to the beads (usually 100 µl is fine).

3) Incubate at 37°C for 5 hours to overnight with shaking at 1250 rpm.

4) Stop trypsin digestion by acidifying to a final concentration of 1% FA and centrifuge at 2,000 x g for 3 min.

5) Transfer the supernatant to a new 1.5 ml protein lobind tube.

9) Resuspend beads in 100 µl of a 60% CH_3CN)/ 1% FA solution, incubate at room temperature for 5 min with shaking at 1250 rpm.

10) Centrifuge at 2,000 x g for 3 min and transfer this second supernatant with the first supernatant.

11) Dry samples in the speed vac.

12) Resuspend peptides by vortexing in 300 µl of a solution of 2 M urea, 1 M NH_4HCO_3, 20 mM HEPES pH 8.0) (in MS-grade water).

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

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

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

16) Desalt on ZipTip (100 µl size).

Adapted from : plateforme protéomique CHU de Québec.