

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.

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 60°C for 30 minutes with shaking at 1250 rpm.
- 3) After a quick spin and cooling, add an equal volume of 15 mM chloroacetamide (CIAA) 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 μl (total) is fine) to quench the CIAA 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 lysis 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 μ l of 0.1% trifluoroacetic acid (TFA) to desalt on ZipTip (100 μ l size).

Digestion without reduction and alkylation

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/ μ l in 20 mM NH_4HCO_3) to the beads (usually 100 μ l is fine).

3) Incubate at 37°C for 5 hours.

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 μ l 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 in 300 μ l of 0.1% trifluoroacetic acid (TFA) to desalt on ZipTip (100 μ l size).

Adapted from : plateforme protéomique CHU de Québec.