On-Beads Trypsin Digestion

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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. However, there are several reports indicating that antibodies which have not been reduced may be partially resistant to trypsin, which could help reduce the amount of antibodies in the sample.

1) Wash beads 5 times with 20 mM ammonium bicarbonate
2) After the final wash step, add enough of 10 mM DTT in 20 mM ammonium bicarbonate to the beads for complete immersion, mix and incubate at 60 °C for 30 minutes.
3) After cooling, add an equal volume of 15 mM iodoacetamide in 20 mM ammonium bicarbonate to the DTT/bead suspension, mix and incubate in the dark for 1 hour.
4) Add 1M DTT to increase the concentration to 15 mM to quench the iodoacetamide and wait 10 minutes.
5) Add 50 ng of trypsin to the beads.
6) Incubated at 37°C for 5 hours to overnight.
7) Stop trypsin digestion by acidifying to a final concentration of 1% formic acid.
8) Harvest the supernatant and transferred to a clean, protein lo-bind tube.
9) Resuspend beads in 60% ACN – 0.1 FA at room temperature for 5 min.
10) Transfer this second supernatant with the first supernatant.
11) Dry samples in the speed vac.
12) Resuspend in 20µl sample buffer (0.1% TFA) to desalt on ZipTip

Digestion without alkylation

1) Wash beads 5 times with 20 mM ammonium bicarbonate.
2) Remove as much wash buffer as possible, beads can be kept at -20°C until use.
3) Add an equal bead volume of diluted trypsin (1ng/µl) to the beads.
4) Incubated at 37°C for 5 hours.
5) Stop trypsin digestion by acidifying with 1% formic acid solution.
6) Harvest the supernatant and transferred to a clean, protein lo-bind tube.
7) Resuspend beads in 60% ACN – 0.1 FA at room temperature for 5 min.
8) Transfer this second supernatant with the first supernatant.
9) Dry samples in the speed vac.
10) Resuspend in 20µl sample buffer (0.1% TFA) to desalt on ZipTip

Adapted from plateforme protéomique CHU de Québec.