In-Gel Digestion Protocol

Sara ten Have 2008

N.B : The volumes given in this protocol are applicable for a single gel band. Upscaling will be required for larger amounts of gel.

1. Wash gel pieces with 100µl 100mM NH_4HCO_3:100%ACN for 10mins, at room temp on a shaker. Remove solution from gel pieces and discard. Repeat.
2. Add 50µL of 100% ACN and watch the gel pieces turn white and aggregate.
3. Add 50µL of 100mM NH_4HCO_3 to make 100mM NH_4HCO_3: 100% ACN. Incubate at 37°C for 30 mins, on a shaker.
4. Remove the solution and dry the gel pieces completely in a vacuum centrifuge. Maximum temperature of 45°C.
5. To the completely dried gel pieces add 50µL 10mM DTT solution and incubate at 55°C for 45 mins in a heated shaker.
6. Remove the DTT solution and add 50µL of 55mM iodoacetamide solution and incubate at room temperature, in the dark for 30mins.
7. Remove iodoacetamide solution and wash gel pieces with 100mM NH_4HCO_3/ 100%ACN 2x 10mins, on a shaker (as with step 1).
8. Dry gel pieces completely in a vacuum centrifuge. Maximum temperature of 45°C.
9. Dilute 10µl of 1µg/µl trypsin in 490µl of 50mM NH_4HCO_3 (or other digestive enzyme). This will result in a pH8 solution (optimal for digestion). Add 10-20µL of diluted trypsin solution to dry gel pieces (ensure gel pieces have rehydrated and have a small amount of additional liquid over the gel pieces to stop drying out). Seal the tubes with parafilm to prevent evaporation.
10. Incubate the gel pieces over night at 37°C.
11. To the gel pieces and trypsin mixture add 20µL of 0.1% TFA and 20µL of 100% ACN. Sonicate in a sonication bath in ice water for 15mins.
12. Remove the supernatant and place in a new eppendorf. Add 100µl 30%ACN:0.1%TFA to the gel pieces. Sonicate as before.
13. Remove the supernatant and add to previous supernatant. Add 100µL of 50%ACN:0.1%TFA to gel pieces and sonicate as before and add supernatant to pooled supernatant.
14. Reduce the volume of pooled supernatant to approximately 100µl in a vacuum centrifuge at 60°C.
15. Clean samples with C18 column (see C18 Column Cleanup protocol).