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In-tube Digestion

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*Starting up (20 µg)

1. Add 11.5 µl of 40% Acr\Bis.

2. 0.4 µl 10% APS.

3. 0.4 µl 10% TEMED.

4. Tap-spin down and leave for 30 min.

5. Fix the protein with fixing solution 500 µl (50% Methanol, 12% Acetic acid) for 30 mins.

6. Remove the gel and cut into pieces.

7. Wash with 50mM Ammonium Bicarbonate, 50% ACN (15 mins/3 times).
(0.19 gm ABC + 25ml CAN + 25ml H_2O)

8. Discard the solution and speed vac (until it dry and become white).

9. For reduction, add 10mM DTT to the gel and incubate at 60 C^0/ 30 mins (in 50mM ABC).

10. For Alkylation, add 55mM IAA to the gel in dark place for 30 mins (in 50mM ABC).

11. Discard the solution and put the plate 25mM ABC drops and cut into pieces and add 200ul on Eppendorf of 25mM ABC.

12. Discard the ABC and dehydrate using 500ul of ACN (10-15 min) (pipette several times).

13. Discard ACN Speed Vac.

14. Digest the sample with 10ng/ µl Trypsin dissolved in 25mM ABC, incubate overnight.
*Starting from this step don't discard the solution and collect it all in new Eppendorf

15. Add 80ul from extraction buffer on the sample (x2).

16. Put the solution in another Eppendorf and add 60-80ul of ACN and put on shaker for 15 min until it become white and dry.

17. Speed vac this solution and reconstitute in 50 µl 0.2% Formic acid.

18. Stage tip.

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