مؤسسة مستشفى سرطان الأطفال-مصر
57357
Children's Cancer Hospital Foundation - Egypt

In-Gel Digestion

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A. Gel Preparation

1. Remove the gel from cassette and add fixing solution (50% Methanol, 12% Acetic acid).
2. Cut each lane into 5 pieces, put each piece in labeled Eppendorf and wash each by 200ul with 50mM ammonium bicarbonate, 50% ACN (15 min/3 times) on shaker.
3. Discard the solution and vacuum dry the sample (till it becomes shrinkage and white colored)

B. Reduction

4. Add 10mM DTT to the gel matrix and incubate at room temperature for 30 min.

C. Alkylation

5. Add 55mM IAA to the gel in dark for 30 min.
6. Discard the solution and cut the pieces into smaller ones in the same Eppendorf and add 25mM ABC/ 15 min on shaker.
7. Discard the solution and dehydrate using 500ul of ACN for 10-15 min (until it gets white and shrinkage).
8. Discard the solution and speed vac for 30-60 minutes until it gets dry.

D. Trypsinization

9. Add 50 ul Trypsin containing 10 ng/ul procaine enzyme.
10. Incubate overnight at 37°C with shaking at 600 rpm.
11. Extract the peptides by adding 80ul of (ACN: milliQ: FA) (66:33:1), respectively shake for 5min. (two times)
12. Transfer the solution in new Eppendorf and each following step will be added in this Eppendorf.

13. Add 80ul ACN to the gel and shake for 15 min (two times) and transfer on the above solution.
14. Take the solution Eppendorf and speed vac.
15. Reconstitute in 50ul of 0.2% F.A.

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