In gel Digestion protocol

Bath at 37ºC
Clean with ethanol the surface before use the gel
50%(v/v) acetronitrile: 25ml acetronitrile + 25ml mqH_2O.
50ml 25mM NH_4HCO_3: 98.82mg up to 50ml with mqH_2O.
12.5ug/ml Trypsin: 20ug in 1.6ml 25mM ammonium bicarbonate.

1. Cut 10 portions
2. Add 50ul of acetronitrile to the gel piece (15min).<Dehydration step and clean the SDS and Comassie, gel pieces will shrink and may become opaque>.
3. Remove the supernatant and rehydrate the spots for 10min with 25ul of 25mM NH_4HCO_3.
4. Remove the supernatant, and then repeat the dehydration step by adding 50ul acetronitrile 15min at RT.
5. Repeat step 3 and 4 two times, to give a total of 3 washes.
6. Remove all liquid and dry spots in speed-vac 10min.
7. Resuspend the dried spots in 10ug/ml trypsin in 25mM ammonium bicarbonate on ice.
   NOTE: <Use 5ul for small gel pieces and 10ul for larger gel pieces. Leave for 5min, the gel should be (nearly) fully rehydrated. If required, add 1-2ul more>
8. Cover gel pieces with 25ul of 25mM Ammonium bicarbonate and incubate at 37ºC 12-16h (o/n).
9. Take the supernatant and keep it in a new eppendorf (NE).
10. Add 50ul 25mM Ammonium bicarbonate **to the gel eppendorf** vortex 4s leave 20min at RT.
11. Take supernatant and keep it in a NE.
12. Add 50ul 2:1 (acetronitrile 100% : 25mM NH_4HCO_3) vortex 4s and leave 20min.
13. Take supernatant and keep it in a NE.
14. Add 50ul 9:1 (100% Acetronitrile : propanol) vortex 4s and leave 20min.
15. Take supernatant and keep it in a NE.
16. Dry down supernatant in NE.
17. Give samples to Spect-Mass department.