In-Gel Trypsin Digestion of Proteins and Peptide Extraction (for Coomassie-stained gels)

1. Before excising bands wash gels in MilliQ for 15'.
2. Excise bands, cut as close to the bands as possible to minimize excess gel material, place in Eppendorf tubes.
3. Add 10mM DTT (dilute DTT 1:10 in 50mM NH_4HCO_3) and incubate for 60' at 56°C
4. Remove DTT and add 50mM Iodacetamid (dilute Ioda 1:10 in NH_4HCO_3) and incubate for 60' at RT
5. Wash gel pieces with 50 – 100 µl MilliQ
6. Wash gel pieces with 50 – 100 µl 50mM NH_4HCO_3 (30').
   Pull off solution and dehydrate with 3/2 Acetonitrile/MilliQ.
7. Repeat step 6. until gel pieces are colourless.
8. Pull off solution and add pure Acetonitrile (10') and air dry to complete dryness.
9. Reswell gel pieces at 4°C on ice for 45' in cold buffer containing trypsin and 50mM NH_4HCO_3 (10.0ng/µl) freshly prepared. The gel pieces should just be covered.
   Add more solution if pieces absorb all of solution.
10. Pull off solution and discard, add the same buffer without trypsin (enough to cover gel pieces) and incubate overnight at 37°C.
11. Collect supernatant, extract peptides by adding 3/2 Acentonitrile/0.1% TFA in MilliQ (60') at room temperature.
12. Collect elution, then cover gel with acetonitrile and incubate for 15' at room temperature
13. SpeedVac dry the combined washes/elutions