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

1. Protein spots are excised, placed in Eppendorf tubes, washed with MilliQ and shrunk by dehydration in acetonitrile
2. Gel pieces are dried in vacuum centrifuge
3. Gel pieces are completely destained in 30 mM $K_3[Fe(CN)_6]$ / 100 mM $Na_2S_2O_3$: 1/1 for 10' and washed afterwards with MilliQ
4. Gel pieces are shrunk again by addition of acetonitrile and dried in vacuum centrifuge
5. Gel pieces are swollen in a digestion buffer (12.5ng trypsin/µl 50 mM NH_4HCO_3) at 4 ^{0}C (on ice)
6. After 45' the supernatant is replaced with 50 mM NH_4HCO_3
7. Enzymatic cleavage : 37 ^{0}C, overnight
8. Peptide extraction : 1/3 20mM NH_4HCO_3, 2/3 acetonitrile (5 h)