Acetone precipitation and protein digestion

1. Chill good quality acetone at -20°C for at least overnight.
2. Add 4X volume of -20°C-acetone to the protein sample solution, mix by vortexing, and allow the proteins to be precipitated for at least 2 hr at -20°C. Overnight incubation is recommended.
3. Centrifuge the precipitated mixture at 16,000 x *g* at 4°C for 10 min.
4. Carefully remove the supernatant.
5. Wash the pellet with 1 mL of -20°C-acetone, mix by vortex, and centrifuge at 16,000 x *g* at 4°C for 5 min.
6. Remove the supernatant and allow the pellet to be air-dried.
7. Add 50 µL of a buffer, which contains 50 mM ammonium bicarbonate (ABC) and 8 M urea, to resuspend the pellet.
8. Add 0.5 µL of 1M DTT into the mixture and incubate at RT for 30 min.
9. Add 2.5 µL of 1M iodoacetamide (IAA) into the mixture and incubate at RT for 30 min in the dark.
10. Dilute the samples with 200 µL of 50 mM ABC.
11. Add trypsin (or other appropriate protease) to digest the protein sample. For trypsin digestion, a ratio of 1:20 to 1:50 (trypsin:protein) is recommended.
12. Incubate the mixture at RT for overnight with rotation.
13. Desalt the peptides by StageTip or ZipTip.