FASP Protocol[1]

1. Mix 30µl of sample with 200µl of 8M Urea in 0.1M Tris:HCl, pH 8.5 in a C18 spin column cartridge and centrifuge for 15mins at 14,000 x g.

2. Add a further 200µl to the C18 spin column and centrifuge again, as before.
3. Discard the flow-through.
4. Add 100µl of 0.05M IAA (IAA should be solubilized in 8M Urea in 0.1M Tris:HCl, pH 8.5) and mix on a shaker at 600rpm for 1 min.
5. Incubate for 20mins at 25°C.
6. Centrifuge for 10mins at 14,000 x g.
7. Add 100µl of 8M Urea in 0.1M Tris:HCl, pH 8.5 to the C18 spin column and spin for 15mins at 14,000 x g. Repeat this step a further x2.
8. Add 100µl of 0.05M NH_4HCO_3 and spin as before. Repeat this step a further x2.
9. To the spin column add 40µl of 0.4µg/µl trypsin solution (trypsin should be diluted with 0.05M NH_4HCO_3). Incubate overnight at 37°C.
10. Remove old collection tubes from column and replace with fresh ones before centrifuging columns for 10mins at 14,000 x g.
11. Add 40µl of NH_4HCO_3 and centrifuge as before.
12. Acidify sample using CF_3COOH and desalt before M/S submission.

1. *Universal sample preparation method for proteome analysis. Wiśniewski JR, Zougman A, Nagaraj N, Mann M. Nat Methods. 2009 May;6(5):359-62. Epub 2009 Apr 19.*
PMID: 19377485