Trypsin Digest Protocol for MS Sample Preparation

Abbreviations used:
2-DE: Two-dimensional gel electrophoresis
ABC: Ammonium bicarbonate
ACN: Acetonitrile
DTT: DL-Dithiothreitol
FA: Formic acid
IAA: Iodoacetamide

Reagents needed:
Ammonium Bicarbonate: Sigma #09830
DTT: Sigma #43817
IAA: Sigma #I1149
Water: Water LCMS Chromasolv: Sigma #39253 or good MilliQ water
Acetonitrile: Acetonitrile LCMS Chromasolv: Sigma #34967
Trypsin: Sequencing grade trypsin: Promega #PRV5111

Solutions needed:
50 mM Ammonium bicarbonate: Dissolve 0.08 g ammonium bicarbonate in 20 ml MilliQ water (make fresh before use every time)

50% Acetonitrile (ACN): 5 ml ACN + 5 ml MilliQ water
70% Acetonitrile (ACN): 1400 µl ACN + 600 µl MilliQ water

10 mM DTT: 0.031 g in 20 ml 25 mM ammonium bicarbonate (make fresh before use)
55 mM IAA: 0.2 g in 20 ml 25 mM ammonium bicarbonate

Trypsin:
Dissolve a single vial of trypsin (20 µg) in 200 µl of the re-suspension solution provided with the trypsin. Divide into aliquots of 20 µl each and store at -80°C. For experimental use: take an aliquot from -80°C and add 180 µl of the 50 mM ammonium bicarbonate for active trypsin. Remember that you cannot re-use this trypsin, it must be discarded after use and made just before use. Keep one ice.

If you have done 2-DE in which you have used DTT and IAA already then steps 16-23 are not necessary.

Preparation of the gel bands or spots:
Gel bands or spots can be dried and stored at -20°C until use.

1. Cut out bands on clean surface with clean scalpel.
2. Cut into small pieces
3. Add 200 µl water and incubate for 10 min, remove the water after 10 min, repeat twice
4. Add 200 µl 50% ACN, 10 min
5. Remove ACN
6. Add 200 µl 50 mM ABC, 10 min
7. Remove ABC
8. Add 200 µl 50% ACN, 10 min
9. Remove ACN

10. Add 200 µl 50 mM ABC, 10 min
11. Remove ABC
12. Repeat until the blue colour is not that strong anymore
13. Add 100 µl 100% ACN until pieces turn white (10 min)
14. Remove ACN
15. Dry in speedyvac

If you have done 2-DE in which you have used DTT and IAA already, then steps 16-23 are not necessary.

16. Add 100 µl 10 mM DTT for 30 min at 37°C
17. Remove DTT
18. Add 200 µl 50 mM ABC, 1 min
19. Remove ABC
20. Add 200 µl 50% ACN, 1 min
21. Remove ACN
22. Add 100 µl 55 mM IAA for 60 min in dark
23. Remove IAA
24. Add 200 µl 50 mM ammonium bicarbonate (ABC), 10 min
25. Remove ABC
26. Add 200 µl 50% ACN, 20 min
27. Remove ACN
28. Dry in speedyvac to remove ACN (± 1 hr)
29. Prepare trypsin by adding 180 µl 50 mM ABC to 20 µl trypsin aliquot. Keep on ice
30. To activate trypsin before addition to the gels pieces incubate at 30°C for 15 min

31. Add 20 µl trypsin to dried gel pieces. It is important that the trypsin just covers the gel pieces, excess trypsin will result in autolysis.
32. Incubate at 37°C for 17 hours.

To extract peptides from gel pieces:

33. Add 15 µl 70% ACN, 30 min
34. Remove supernatant to clean eppendorf tube (NB to pool all the supernatant into the same tube)
35. Add 15 µl 70% ACN, 30 min
36. Remove supernatant to clean eppendorf tube (NB to pool all the supernatant into the same tube)
37. Dry supernatant in speedyvac (dry sample should be stable for ±2 months)

The dried peptides can then be brought to the Proteomics Unit for MS analysis. If you suspect that your sample has particles or possible contaminants it can be cleaned further by using a Zip-tip (C18-tips that can be obtained from Merck, Sigma and Waters).