MSU Proteomics Manual Digestion Procedure

Modified from Jensen, et.al., Methods in Molecular Biology, Vol.112, 1999 based on Shevchenko, Anal Chem. 1996 Mar 1;68(5):850-8

Stock Solutions:

*- Note: procedure is written with volumes generally needed for ~20 2D gel spots. Stock volumes may need to be increased to accommodate more samples and experimental volumes may need to be increased so that the added solutions completely cover the gel pieces.

100mM Ammonium Bicarbonate (Ambic)

Weigh out 79mg of ambic and dissolve in 10 mL ultrapure water.

10 mM Dithiothreitol (DTT)

Weigh out 1.54mg of Dithiothreitol into an eppindorf tube and dissolve in 1 mL of 100mM ambic.

55 mM Iodoacetemide (IAA)

Weigh out 10.18mg of Iodoacetamide into an eppindorf tube and dissolve in 1mL of 100mM ambic.

Resuspension Buffer

50mM Ambic in ultrapure water. You should have ample 100mM ambic so simply dilute an appropriate amount 1:1 in ultrapure water.

Digestion Buffer

Resuspend 20ug of lyophilized trypsin in 200uL of resuspention buffer. Take 15ul of this and add 100ul of 50 mM ambic to give a final trypsin concentration of 15ng/uL.

100% Acetonitrile

HPLC grade or better

Day 1 – Prepare the Samples and begin digestion

Prepare the Gel Pieces

1. Pour out the stain and replace with destain solution and return to the shaker. Perform 2 to 4 washes until the staining is sufficiently low.

2. Select bands from the sample lane and remove them one at a time using a razor blade. Slice the excised band into 1-2 mm sections and place the pieces into a labeled tube. Adding ~100uL of water or Ambic to the tube will make it easier to place the gel pieces inside.

3. Prepare the ambic and DTT solutions for the digestion procedure. Weigh out the IAA. Do not reuse any of these solutions make them fresh for every digestion.

In-gel Digestion

1. Wash gel pieces with 100mM ambic (~100uL) for 5 min.

2. Discard buffer.

3. Add 50uL 100% Acetonitrile and dehydrate the gel pieces at RT. Do several exchanges (3-4) of Acetonitrile for 5-10 min each until you see the gel pieces have shrunken and appear chalk white.

4. Rehydrate the gel pieces with 50uL of 10 mM DTT in 100 mM ambic.

5. Heat @ 56°C for at least 45 min to reduce the sample.

6. Decant DTT solution.

7. Add 100% acetonitrile and incubate at RT. Repeat as in step 3 until the gel is completely dehydrated.

8. Add 50uL of 55mM IAA in 100mM ambic to alkylate cysteines.

9. Incubate for a minimum of 20 min in the dark at RT.

10. Discard supernatant and wash briefly with ~50 uL of 100mM ambic.

11. Replace with 100 mM ambic and wash for 15 min at RT. If the gel is not completely destained, wash with 1:1 acetonitrile:100mM ambic at 37°C for 30 min.

12. Decant liquid and add 50uL 100% acetonitrile. Dehydrate gel pieces as before.

13. Rehydrate in approximately 20uL digestion buffer. If needed add more so that gel pieces are completely covered.

14. Incubate at 37°C overnight.

Day 2: Finish digestion

Stock Solutions:

60%Acetonitrile(ACN)/1% Trifluoroacetic Acid(TFA) (v/v)

HPLC grade or better in ultrapure water.

2% ACN/0.1% TFA (Blank solution) (v/v)

HPLC grade or better in ultrapure water.

In-gel Digestion

15. Spin down the sample in the desktop centrifuge for ~2 min to force any liquid to the bottom of the tube.

16. Collect the liquid in a new, labeled 1.5mL eppindorf tube and set this aside either on ice or in the refrigerator.

17. Add 50 uL of 60%ACN/1%TFA to each gel piece and sonicate for ~5min.

18. Spin down the tubes and collect supernatant. Add to the liquid collected in step 16. Repeat twice for a total of 3 extractions.

19. Dry the collected samples in the Speedvac until ~ 2uL remains. Be careful not to overdry the samples. Save the now depleted gel pieces in the refrigerator.

20. Add ~18 uL of 2%ACN/0.1%TFA solution (Blank solution) to the tubes making the final volume 20uL.

21. Sonicate the tubes for ~5 min.

22. Spin down the samples in the desktop centrifuge and store at 4^0C if samples can be analyzed in the next 24hrs, otherwise store at -20^0C.