

Modified from: Shevchenko, A., Wilm, M., Vorm, O., Mann, M., *Anal Chem.* 1996 Mar 1;68(5):850-8.

Solutions:

100mM Ammonium Bicarbonate (Ambic) – 80mg Ambic into 10mL ultrapure water; pH should be ~8 but do not adjust

100% Acetonitrile (ACN)

10mM Dithiothreitol (DTT) – 1.54mg DTT into 1mL 100mM Ambic

55mM Iodoacetamide (IAA) – 10.18mg IAA into 1mL 100mM Ambic

50mM Ammonium Bicarbonate (Ambic) – dilute remaining 100mM Ambic 1:2 with ultrapure water

Digestion Buffer – dissolve trypsin stock powder (20ug) in 200uL 50mM Ambic giving a 0.1ug/uL stock solution. Take 5uL of this stock and add 100uL of 50mM Ambic to create 5ng/uL working solution

Extraction Buffer – Make a solution of 60%ACN, 1% Trifluoroacetic Acid (TFA) in water

Blank Solution – Make a solution of 2% ACN, 0.1% TFA in water

- 1) Chop gel slices into ~1mm X 1mm cubes with an ethanol cleaned razor blade and place the pieces into an eppendorf tube. If you add ~100-200uL of Ambic to the tube prior to placing the cut gel into it this will make the pieces easier to load into the tube.
- 2) Wash gel pieces with 100mM Ambic for ~5min. Discard Ambic.
- 3) Add enough ACN to the gel pieces so that they are completely submerged, usually takes ~100uL, and incubate for 5min. Remove the ACN and repeat. Continue exchanges until you see the gel pieces shrink and turn chalk white.
- 4) Remove ACN and replace with 10mM DTT solution. Again, use enough to completely submerge the gel pieces. Generally this will be 100uL-200uL but using more is fine. Incubate the tubes at 56°C for 45min.
- 5) Remove DTT solution and replace with ACN. Repeat step 3.
- 6) Remove ACN and cover gel pieces with 55mM IAA solution. Incubate at room temp. in a dark drawer for 20min.
- 7) Remove IAA and replace with 100mM Ambic. Incubate for ~5min to remove residual IAA.
- 8) Remove Ambic and replace with ACN. Repeat step 3.
- 9) Remove ACN and replace with digestion buffer. Add enough buffer to ensure that the gel pieces will be completely covered when rehydrated but with as little excess buffer as possible. This is a bit of a guessing game but use the volumes you used for DTT and IAA to help estimate what you need. If you know the total amount of protein present in the sample add trypsin at a ratio of 1:100 otherwise use the recipe above. Incubate at 37°C overnight.
- 10) The following morning, spin the tubes briefly in the centrifuge and collect any remaining digestion buffer from the samples into new eppendorf tubes and set these aside. Submerge gel pieces in extraction buffer and sonicate in the water bath for ~30sec.

- 11) Spin the tubes in the centrifuge briefly and remove the extraction buffer. Add this to what you collected in step 10. Repeat the extraction and add this to what you've already collected.
- 12) Dry the extracted peptides in the SpeedVac until ~1uL remains.
- 13) Store dried peptides at -20C or if you will proceed directly to MS analysis, add 20uL of Blank solution to each sample and sonicate for ~30sec.