August 31st, 2006 (Scott Hsieh)

Trypsin In-Gel Digest Protocol

Preparation of reagents

100 mM NH_4HCO_3, pH 9.2
Simply dissolve 0.079 g of NH_4HCO_3 in DI water to a volume of 10 mL. The pH should be around 9.2 after doing this so adjustment is not needed.

10 mM DTT in NH_4HCO_3 (1.543 mg/mL)
Because of the small amounts of DTT needed to make 10 mM DTT solution it is recommended to make a 1 M DTT stock, which can then be diluted to the needed concentration. A suggested way to do this is to tare an empty microfuge tube, add a small amount of DTT, calculate an appropriate volume of NH_4HCO_3 to add for the weight of that small amount, then resuspend the DTT in that volume. Make this solution fresh each time you use it.

50 mM Iodoacetamide (IAA) in NH_4HCO_3 (9.245 mg/mL)
It is recommended to make a 0.5 M IAA (10X or 92 mg/ml)) stock, which is then diluted to the needed concentration. You should make this solution fresh each time you need it.

CH_3CN
I usually use CH_3CN from EMD (AX0151-1)

2% Trifluoroacetic Acid (TFA)
I normally use TFA from Pierce (28903)

10 ng/µL Promega sequencing grade, modified trypsin (Cat no. V5113)

NOTE: Volumes given in this protocol are APPROXIMATE. The size of the gel slice will determine how much of each reagent is actually needed. A good rule of thumb is to simply make sure that the solution volume covers at least 75% of the area of the gel. Also it is recommended to work in **500 µL** tubes. Also for steps that require using a speedvac, **do NOT use the speedvac in 5043**. (Acid in samples will ruin pump.) You may use the speedvac in the common area near the - 80˚C freezer.

Washing
1) If applicable, transfer gel slices into 500 µL tubes before starting. This should reduce the volumes of reagents needed for this protocol.

2) If gel slices are dried, allow them to swell for approximately 10 mins in 30-50 µL of 100 mM NH_4HCO_3.

3) Soak gel slices in 100 µL of a 50:50 mix of 100 mM NH_4HCO_3/CH_3CN. Let stand for 10 mins.

4) Discard supernatant and add 30 µL CH_3CN. Let stand for 10 mins. Discard supernatant.

5) Repeat steps 2 and 3 twice or until gel is destained.

6) Dry the gel slice in speedvac for 5-10 mins. **Do NOT use the speedvac in 5043**.

Reduction of disulfides and blocking of free thiols

1) Add 20 µL of 10 mM DTT to gel slice. Incubate at 60˚C for 1 hour on a heating block.

2) Allow samples to cool to room temperature and discard supernatant.

3) Add 20 µL of 50 mM iodoacetamide. Allow samples to incubate in the dark for 45 mins at 45 ˚C. Discard supernatant.

Note: If using volumes different from those given, DTT and iodoacetamide should be added in equal volumes.

Wash gel slices

1) Add 50 µL of 100 mM NH_4HCO_3 and let stand for 10 mins.

2) Discard supernatant and add 50 µL CH_3CN. Let stand for 10 mins then discard supernatant.

3) Repeat steps 1 and 2.

4) Dry the samples in the speedvac for 5-10 mins. **Do NOT use the speedvac in 5043**.

Trypsin digest

1) Swell the gel slices in 10 µL trypsin (refer to step above) and let sit in an ice bath for 45 mins. (Do not worry about covering the gel slice in this step)

2) Add 10-20 µL of 100 mM NH_4HCO_3, and digest overnight at 37 ˚C. (Do not allow digestion time to exceed 24 hrs.)

3) Add 20 µL of H_2O to each sample and let stand for 5 mins. Transfer supernatant to a new tube.

4) Add 30 µL of 50% CH_3CN/1% TFA to each gel and let stand for 10 mins. Add this new supernatant to the supernatant previously collected.

5) Repeat step 4 two more times.

6) Dry the extracts in the speedvac. (This may take several hours depending on your sample, but be sure not to over dry your extracts.) **Do NOT use the speedvac in 5043**.

7) Store protein at 4˚C either dry or resuspend in appropriate buffer dependent on what type of analysis you want to do.

Note: It is recommended that you initially resuspend pellets from step 7 in a buffer containing 50% CH_3CN/ 1% TFA and dry down again to further clean the sample. Then resuspend in buffer that is appropriate for analysis.

For LC-MS/MS: 0.1% Formic acid
For Nanospray MS/MS: 50% CH_3CN/ 2% Formic acid
For MALDI: 50% CH_3CN/ 1% TFA