

TRYPsin DIGESTION OF PROTEIN SOLUTIONS

From Univ. of Arizona, Center for Toxicology, Proteomics Core
http://swehsc.pharmacy.arizona.edu/analysis/Solution_Digest.PDF

Dialysis (If necessary to clean up sample)

- Make dialysis buffer, 0.2M NH_4HCO_3 , pH ~ 8.00.
- Use 8000 M.W.C.O., cut a 3.5-inch piece of dialysis membrane and cut open along seam (for Mini-micro dialyzer) or use premade dialysis frames for Micro-dialyzer.
- Wash dialysis tubing thoroughly with ddH₂O.
- Fill the dialysis unit with dialysis buffer and secure the dialysis membrane in place.
- Place the dialysis unit on stir plate, slowly stir.
- Dispense protein solutions into wells and dialyze for a minimum of 3 hours.
- After dialysis, dispense equal amounts to the protein solutions into clean 1.5mL microcentrifuge tubes.
 - These solutions can be frozen at -20°C, indefinitely for use as digestion controls.

Digestion

- Make Trypsin Solution to a concentration of [0.1 $\mu\text{g}/\mu\text{l}$], 1 hour before use and place on ice.
 - If proteins have disulfide bonds, a reduction/alkylation process will be necessary prior to trypsin addition.
 - Add 40 μl DTT solution and incubate at 56°C for 45 mins.
 - Remove samples from heat and allow to cool to room temperature
 - Add 40 μl of 55mM iodoacetamide solution and incubate at room temperature for 30 mins, in the dark.
- Add trypsin to each tube at a 1:50, (protease:protein) ratio, close the tube and gently flick to mix.
- Place the samples in 37° C water bath for 2 hours.
- After 2 hours add an additional amount of trypsin to each sample, equal to the first addition.
- Continue to incubate at 37° C for 17-18 hours.
- Add 10 μl of Formic Acid to solution. Alt: Add 10 μl of 10% TFA to solution
- Store at -20° C.

Ammonium bicarbonate (NH_4HCO_3) for dialysis buffer.

Fisher Scientific Cat. #A6430-500

Hydrochloric acid

Malinckrodt, AR Cat. #2612

Concentrated 12N

Apomyoglobin

Sigma Cat. #A-8673

Diluted with 1mL of 1:1 MeOH:H₂O with 0.5% AcOH

Bovine Serum Albumin

Sigma Cat. #A-7030

Mixed with ddH₂O to desired concentration.

Trypsin Modified sequencing grade: Dilute to 0.1 $\mu\text{g}/\mu\text{l}$ by adding 200 μl of buffer to vial.

Promega Cat. #V5111 E.C. 3.4.21.4

Sigma Cat. #T6567, Proteomics Grade Trypsin

Iodoacetamide (mix just prior to use)

10mg Iodoacetamide + 100 μl 1M Ambic + 900 μl ddH₂O

DTT solution (mix just prior to use)

5 μl 2M DTT stock + 895 μl ddH₂O + 100 μl 1M Ambic

2M DTT stock solution

154.3mg DTT/500 μl ddH₂O (can be stored at -20 for 4weeks.)