

## TRYPsin DIGESTION OF PROTEIN SOLUTIONS

### Digestion

- 1) Make Trypsin Solution to a concentration of [0.1ug/ul], 1 hour before use and place on ice.
  - a) If proteins have disulfide bonds, a reduction/alkylation process will be necessary prior to trypsin addition.

Note: The next steps 1 i) –iii) need to be performed in the dark.

  - i) Add 40ul DTT solution and incubate at 56 C for 45 mins.
  - ii) Remove samples from heat and allow to cool to room temperature
  - iii) Add 40ul of 55mM iodoacetamide solution and incubate at room temperature for 30 mins, in the dark.
- 2) Add trypsin to each tube at a 1:50, (protease:protein) ratio, close the tube and gently flick to mix.
- 3) Place the samples in 37 C water bath for 2 hours.
- 4) After 2 hours add an additional amount of trypsin to each sample, equal to the first addition.
- 5) Continue to incubate at 37 C for 17-18 hours.
- 6) Reduce volume to approx. 10 ul in the speed-vac.
- 7) Store at -20 C.

### Trypsin Modified sequencing grade

Promega Cat. #V5111 Dilute to 0.1 ug/ul by adding 200ul of buffer to vial.  
E.C. 3.4.21.4

### Iodoacetamide (mix just prior to use)

10mg Iodoacetamide + 100ul 1M Ambic + 900ul ddH<sub>2</sub>O

### DTT solution (mix just prior to use)

5ul 2M DTT stock + 895ul ddH<sub>2</sub>O + 100 ul 1M Ambic

### 2M DTT stock solution

154.3mg DTT/500ul ddH<sub>2</sub>O (can be stored at –20 C for 4weeks.)

### 1M Ammonium Bicarbonate (Ambic)

7.906g/100ml ddH<sub>2</sub>O

06/28/04