

## IN-SOLUTION DIGESTION PROTOCOL

### DIGESTION PROCEDURE

1. Reconstitute sample in approximately 20  $\mu\text{L}$  of 8.0 M urea in a 0.5 mL microfuge tube.
2. Add 1  $\mu\text{L}$  of Reducing Reagent and mix the sample by gentle vortex.
3. Reduce the mixture for 1 hour at room temperature or in an oven at  $37^\circ\text{C}$ . Do not go over  $37^\circ\text{C}$  or the urea will react with the sample and generate carbamylated artifacts.
4. Add 20  $\mu\text{L}$  of Alkylating Reagent and alkylate for 1 hour at room temperature in the dark (you can use aluminum foil to cover up the sample).
5. Add 4  $\mu\text{L}$  of Reducing Reagent to consume any leftover alkylating agent (so the trypsin is not alkylated).
6. Add 60  $\mu\text{L}$  of Ambic to dilute the urea before digesting it with trypsin.
7. Add trypsin in appropriate ratio (1:30) to approximate amount of protein by weight. Digest overnight at  $37^\circ\text{C}$ .
8. In the morning, add 1  $\mu\text{L}$  of acetic acid to stop the digestion. Vortex and centrifuge.

### SOLUTIONS FOR IN-SOLUTION DIGESTION

#### **Ammonium Bicarbonate (Ambic)**

1M stock Ambic  $\rightarrow$  0.79 g in 1mL water

100mM Ambic  $\rightarrow$  100 $\mu\text{L}$  stock in 1mL water

#### **8M Urea**

480 mg urea in 1.0 mL of 100 mM Ambic

#### **Reducing Reagent (DTT)**

1M DTT  $\rightarrow$  30 mg DTT in 200  $\mu\text{L}$  of 100 mM Ambic

#### **Alkylating Reagent (Iodoacetamide)**

200mM iodoacetamide  $\rightarrow$  Dissolve 36 mg (0.036 g) in 1 mL of 100 mM Ambic

#### **Trypsin solution**

Trypsin  $\rightarrow$  0.10  $\mu\text{g}/\mu\text{L}$  (in -80 freezer)