Protocol for In-Solution Tryptic Digestion

I. Material Preparation

a. **Trypsin Stock**: Prepare the Trypsin Stock solution in a concentration of 0.1ug/uL by adding 200uL of Trypsin resuspension buffer to 20ug of Trypsin. Store this solution at -20°C for up to two months.

b. **Digestion Buffer**: Weigh 10 mg of the Ammonium Bicarbonate and dissolve in 2.5mL of ultrapure water for a final concentration of ~50mM. This solution can be stored at 4°C for up to two months.

c. **Reducing Buffer**: Weigh 8 mg of DDT and dissolve with 500uL of ultrapure water for a final concentration of ~100mM. Store reducing buffer at -20°C.

d. **Alkylation Buffer**: Prepare Alkylation Buffer just before use. Weigh 9 mg of Iodoaceamide and add it to a foil-wrapped tube to avoid exposure to light. Add 500uL of ultrapure water for a final concentration of ~100mM. Do not store excess.

e. **Protein Concentrations**: The procedure described below has been found to work well with protein concentrations in the range of 0.1 – 1.0 mg/mL. This concentration range will allow you to work with from 1.0ug to 10ug of protein, respectively, in 10uL total volume for the digestions.

II. Procedure for In-Solution Digestion

a. **Reduction and Alkylation**

i. Add 15uL of **Digestion Buffer** and 1.5uL of Reducing Buffer to a 0.5mL microcentrifuge tube.

ii. Add 10uL of the protein solution to the tube and adjust the final volume to 27uL with ultrapure water.

iii. Incubate sample at 95°C for 5 minutes. Allow sample to cool.

iv. Prepare **Alkylation Buffer** as described in the **Material Preparation Section**. Add 3uL of Alkylation Buffer to the tube and incubate in the dark at room temperature for 20 minutes.

b. **Digestion**

i. Prepare Trypsin as described in the Material Preparation Section.

ii. Add 1uL Trypsin to reaction tube and incubate at 37°C for 3 hours.

iii. Add an additional 1uL Trypsin and incubate the reaction at 30°C overnight.

iv. Spot 0.5 µL of α-cyano-4-hydroxycinnamic acid matrix (10 mg/mL in 50% HPLC grade acetonitrile, 0.1 % TFA) on MALDI sample plate followed by 0.5 µL of sample. Unused sample may be frozen at -20°C in case further analyses are required.

v. Allow spots to dry completely and perform MALDI MS data collection as soon as possible.

Notes

- Always use non-latex gloves when handling samples, keratin and latex proteins are potential sources of contamination.
- Never re-use any solutions, abundant proteins will partially leach out and contaminate subsequent samples.

References

1. Procedure modified from Pierce In-Solution Tryptic Digestion Kit.