In-gel digestion

<Notice!> Wearing cleaned gloves is needed, and manipulating in laminar flow cabinet is preferred.

Prepare reagents before use:

- ✓ 25 mM ammonium bicarbonate (ABC) solution (pH 8.5)
- ✓ 25 mM ABC solution/50% ACN solution
- ✓ 0.1% (w/v) trypsin solution: Dilute 1 μL of 12.5 ng/μL trypsin stock in 100 μL 25 mM ABC solution.

Procedure:

Day 1

1. Destain (for silver stain):
   - i. Prepare 0.1 g/mL of sodium thiosulfate ($Na_2S_2O_3$) and potassium ferricyanide ($K_3Fe(CN)_6$).
   - ii. Add 100 μL of 25 mM ABC solution.
   - iii. Add 10 μL of $Na_2S_2O_3$ solution.
   - iv. Add 10 μL of $K_3Fe(CN)_6$ solution.
   - v. Incubate at R/T for 10 min.
   - vi. Spin down gel pieces and remove destain solution.
   - vii. Add 100 μL of 25mM ABC solution and vortex for 10 min. Repeat once.
   - viii. Spin down gel slices and remove liquid.

2. Add 200 μL of 50% ACN/25 mM ABC solution and vortex for 10 min. Remove liquid and repeat once.
3. Dry gel pieces by SpeedVac for 10 min.

4. Reduction
   - i. Prepare 10 mM dithiothreitol (DTT)/25 mM ammonium bicarbonate (ABC) solution: Dissolve 1.5 mg of DTT in 1000 μL of 25 mM ABC solution.
   - ii. Add 100 μL of 10 mM DTT/25 mM ABC solution to each sample.
   - iii. Incubate at 56℃ for 1 hr.
   - iv. Spin down gel slices and remove the DTT solution.

5. Alkylation
   - i. Prepare 55 mM iodoacetamide (IAM)/25 mM ABC solution: Dissolve 10 mg of IAM in 1000 μL of 25 mM ABC solution.
   - ii. Add 100 μL of 55 mM IAM/25 mM ABC solution to each sample.
   - iii. Incubate at R/T in the dark for 30 min.
   - iv. Spin down gel slices and remove the IAM solution.

6. Add 100 μL of 25 mM ABC solution. Vortex for 10 minutes. Spin down gel pieces and remove the buffer solution.
7. Add 100 μL of 50% ACN/25 mM ABC solution. Vortex for 10 minutes. Spin down gel pieces and remove the buffer solution.

8. Dry gel pieces by SpeedVac for 10 min.

9. Add 100 μL 0.1% (w/v) of trypsin solution and incubate at 4℃ for 20 min to rehydrate gel pieces.

10. Incubate at 37℃ for at least 16 hr.

Day 2

11. Add 100 μL of 50% acetonitrile (ACN)/25 mM ABC solution. Sonicate for 1 min. Repeat this step twice.

12. Aspirate the supernatant to new eppendorf.

13. Dry the supernatant by SpeedVac.

14. Redissolve peptide with 20 μL of 0.1% formic acid. About 5 min later, centrifuge at 10,000g for 10 min. (5 ul loading, 11~12 ul resuspension)