Mass Spectrometry

(1) Preparation of chemicals

-Chemicals

Methanol (MeOH) for blotting sequence type

Acetonitrile (CH_3CN) HPLC grade

Trifluroacetic acid (TFA) HPLC grade

-Stock solutions

(Preserve at 4 ^oC)

100 mM NH_4HCO_3

500 mM EDTA.2Na

Washing solution (25% MeOH / 7% Acetic acid)

10 mM Tris-HCl (pH 8.0)

3% TFA

0.1% TFA / 100% CH_3CN

0.1% TFA / 100% H_2O

(Freezer at -20^oC)

1 M Dithiothreitol (DTT)

10 pM Trypsin / 10 mM Tris-HCl

-Chemicals to be prepared during use

a. Destaining solution (50 mM NH_4HCO_3 / 50% MeOH)

100 mM NH_4HCO_3	2.5 ml
100% MeOH	2.5 ml

b. Reduction solution (10 mM EDTA.2Na / 10 mM DTT / 100 mM NH_4HCO_3)

500 mM EDTA.2Na	20 µl
1 M DDT	10 µl
100 mM NH_4HCO_3	970 µl

c. Alkylation solution (10 mM EDTA. 2Na / 40 mM Iodoacetamide / 100 mM NH_4HCO_3)

500 mM EDTA.2Na	20 µl
Iodoacetamide	7.4 mg
100 mM NH_4HCO_3	980 µl

d. Trypsin solution

10 pM Trypsin / 10 mM Tris-HCl	50 µl
10 mM Tris-HCl (pH 8.0)	450 µl

(2) Methods

a. Cut the gel (spot)

Cut the selected protein spots from the 2D-gels. Place the gels on light box and cut the protein spots with a spatula. Collect the gel pieces in an eppendorf tube.

Wash the gel pieces with water (MQ), drain out water. This can be preserved in freezer at –20^{o}C.

b. Washing and destaining

Add 1 ml wash solution (25% MeOH / 7% Acetic acid) to the eppendorf tube, fix them in a rotor and rotate over night at room temperature.

c. Destaining procedure depends on the method of staining:

In case of CBB staining: Wash with water (MQ), add 0.5 ml destaining solution (50 mM NH_4HCO_3 / 50% MeOH), incubate at 40 ^{o}C until the samples (gel pieces) are destained (may take 30 to 60 min). After destaining, dry out destaining solution then wash with water (MQ).

In case of silver staining: Add 0.3 ml solution (20 mM EDTA. 2Na / 50 mM Tris-HCL wait for 5 min at room temperature. Drain out supernatant, repeat this step once more. Then wash with water (MQ) for two times.

d. Reduction and Alkylation

i) Dry the samples in speed Vac. concentrator (may skip this step)

ii) Add 50 µl reduction solution (10 mM EDTA.2Na / 10 mM DTT / 100 mM NH_4HCO_3) to the eppendorf tube to make the gels wet. Incubate for 1 h at 60 ^{o}C placing on a cool block.

iii) Discard the solution and dry again using speed Vac.

iv) Add 50 l alkylation solution (10 mM EDTA.2Na / 40 mM Iodoacetamide / 100 mM NH_4HCO_3) to the gels in eppendorf tube, place in dark at room temterature for 30 min to carry out reaction.

v) Add 1 ml water (MQ), wash the gels and drain the water. Repeat this step for 2 times.

vi) Smear the gels with a Pelletier.

vii) Dry the gels in a speed Vac. concentrator.

e. Digestion

i) Prepare 1 pM trypsin solution (10 pM Trypsin / 10 mM Tris-HCl), add 50 µl to the gels in eppendorf tube.

ii) Wrap the eppendorf tube with Para film, digest at 37 ^{o}C for 10 to 12 h.

f. Elution

i) Add 100 µl solution (0.1% TFA / 100% CH_3CN) to the gels in eppendorf tube, sonicate for 10 min.

ii) Collect the supernatant in a new eppendorf tube.

iii) Add 100 µl solution (0.1% TFA / 100% MQ) to the gels in eppendorf tube, sonicate for 10 min.

iv) Collect the supernatant in eppendorf tube.

v) Add 80 µl solution (0.1% TFA / 50% CH_3CN) in the gels in eppendorf tube, sonicate for 10 min.

vi) Collect the supernatant in eppendorf tube.

vii) Add 100µl solution (0.1% TFA / 100% CH_3CN) to the gels in eppendorf tube, sonicate for 10 min.

viii) Collect the supernatant in a new eppendorf tube.

ix) Concentrate the collected sample to 20 - 30 µl in a speed Vac. (CH_3CN will be evaporated). This sample is peptide solution.

g. Desalt

i) Using reverse phase column, desalt and concentrate the peptide solution.

ii) Wash with ZipTip C18 (Millipore) filling and unfilling 10 µl 0.1% TFA / 50% CH_3CN 3 times

iii) Wash and stabilize ZipTip with 10 µl 0.1% TFA / 100% MQ, filling and unfilling 3 times

iv) Take the whole peptide solution in ZipTip (10 µl each time) in repeated times, thus absorbing the whole peptides in ZipTip columniv) Wash and desalt the ZipTip with 10 µl 0.1% TFA / 100% MQ, repeat 3 times

v) Dissolve the peptides absorved in column by 0.1% TFA / 50% CH_3CN. The sample volume depends on the following operation.

<u>In case of analyzing using VOYAGER-RP:</u>

Use 2 µl peptide on the sample plate and 2 µl Matrix solution, mix them well, air dry and analyze.

<u>In case of analyzing using other mass spectrometer</u>

The sample volume depends on requirement.

<u>In case of preservation for future analysis</u>

Take 5 µl in an eppendorf tube and keep in freezer at -20^{o}C.

(3) Data analysis

For MALDI-TOF analysis, four criteria were used to assign a positive match with a known protein. (i) The deviation between the experimental and theoretical peptide masses should be less than 50 ppm. (ii) At least four different predicted peptide masses needed to match the observed masses for an identification to be considered valid. (iii) The coverage of protein sequences by the matching peptides must reach a minimum of 10%. (iv) The score that was obtained from the analysis with Mascot software indicates the probability of a true positive identification and must be at least 50.