Tryptic Digest of Proteins in Solution

Reagents:

100 mM Ammonium Bicarbonate (ABC):
0,8 mg ABC in 100ml dd water

1 M Tris pH 8:
Taken from media kitchen

1 mg/ml DTT (Dithiothreitol) = 6.5 mM
1 mg of DTT powder (MW=154.253 g/mol, Roche; 10708984001) is dissolved in 1ml 100 mM ABC

200 mM MMTS (methyl methanethiosulfonate):
9 µl of 10,6 M MMTS (Fluka 6430619) + 491 µl Isopropanol (store in -20°freezer)

50 mM acetic acid:
Prepare 1 mol/l acetic acid: 15.8 ml dd water + 1 ml glacial acetic acid (16.8 mol/l) (Merck; 1.000 66.0250)
dilute the 1 mol/l 1:20 with dd water to 50 mM acetic acid

100 ng/µl Trypsin stock solution:
dissolve 100 µg Trypsin (Trypsin Gold, mass spectrometry grade, Promega V5280) in 1 ml 50 mM acetic acid (store at -80ºC)

10% TFA:
9 ml dd water + 1 ml 100% TFA (Thermo Scientific 28903)

Workflow:

The pH of the protein containing sample should be between 7.5 -8.5 to allow an optimal tryptic digest. Use 1M Tris pH 8 to adjust the pH.

For samples contained in a volume below 100µl the following procedure is used:

<u>Reduction with DTT:</u>
Add 2 µl of the 1 mg/ml DTT stock solution to the sample and incubate it for 30 min at 56°C in the thermoshaker at 700 rpm.

<u>Alkylation with MMTS:</u>
Prepare a 40 mM MMTS solution by diluting the 200mM MMTS stock 1:5 in 100mM ABC buffer. Add 2 µl of the 40 mM solution to the sample and incubate it for 30 min at room temperature in the dark.

<u>Digest:</u>

Add 2 µl 100ng/µl trypsin to the sample and incubate it at 37°C for 2 hours. Then add another 2 µl of the trypsin solution and proceed with the incubation at 37ºC over night – (total amount of trypsin added: 400 ng trypsin)
Stop the digest by addition of 10 µl of 10% TFA – pH should be acidic.

Control of digest efficiency:
To control the digest efficiency and evaluate the general sample quality, 5-10% of the digested sample is separated on a monolithic column.
The outcome of the test is the basis for decisions on both the injected amount of the sample and the specific LC-MS/MS method.

Variations to the above protocol

Larger sample volumes:
If the sample is contained in a volume larger than 100 µl, adapt all working steps as follows: For a volume smaller than 150 µl, add 1.5 times the amount of reagent for each step, for a volume between 150 and 200 µl add double amounts.

Digests using other enzymes:
Chymotrypsin: add 400 ng and incubate for 5h at 25°C (or 2x200ng for 2x2,5 hours)
Subtilisin: add 400 ng and incubate for 1h at 37°C
Lys-C: add 2 x 200 ng and proceed as described for Trypsin
Glu-C: add 2 x 200 ng and proceed as described for Trypsin (or incubate at 25 °C)
Arg-C: add 2 x 200 ng and proceed as described for Trypsin

100 ng/µl Chymotrypsin stock solution:
dissolve 25 µg Chymotrypsin (Sequencing grade, Sigma-Aldrich) in 250 µl 1 mM HCl (store at -80ºC)

10 ng/µl Subtilisin stock solution: must be freshly prepared!
Prepare a Urea/Tris dilution solvent by mixing 9 vol. parts of 6 M Urea with 1 vol. part of 1 M Tris (eg.: 1800 µl Urea + 200 µl Tris).
Weigh in 5 mg Subtilisin and dissolve it in 500 µl 1 mM HCl (c = 10 µg/µl).
Dilute the enzyme stock first 1:5 with 1 mM HCl. (c = 2 µg/µl) and subsequently 1:200 with the Urea/Tris dilution solvent (c = 10 ng/µl).
For each 100 µL of your sample, add 40 µL subtilisin solution.

10 µg/µl Lys-C stock solution:
Dissolve Lysyl Endopeptidase (Wako, 129-02451, 10-12 AU/vial) in H_2O to a final conc of 10 µg/µl (store at -80ºC)

1 µg/µl Glu-C stock solution:
Dissolve 50 µg Glu-C (Roche, No 11420399001, 1x 50 µg) in H_2O to a final conc of 10 µg/µl (store at -80ºC)

100 ng/µl Arg-C stock solution:
dissolve 10 µg Arg-C (Sequencing grade, Promega V1881) in 100 µl H_2O (store at -80ºC)