LYS-C OR TRYPSIN IN-SOLUTION PROTEIN DIGEST PROTOCOL

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This protocol can be adapted to use other proteases. Change the buffer from Tris to one that can give a pH appropriate for the desired protease, then follow the trypsin directions.

1. Bring solution up to 8M Urea and 100mM Tris-HCl pH 8.5 (use 10 M urea and 1 M tris stocks; ideal final volume is about 80µl).
2. Add 100 mM TCEP (a reducing agent) to a final concentration of 5 mM. Incubate at room temp. for 20 min.
3. Add 500mM iodoacetamide (make fresh daily) to a final concentration of 10 mM. Incubate at room temp. for 15 min. in the dark (covered with foil).
4. Use one of the following enzymes:

Lys-C Digest:

1. Add in Lyse-C 1µl (0.1µg/µl), 1/100th total amount.
2. Incubate for 4 hr. at 37°C in the dark.

Trypsin Digest:

1. Dilute samples by a factor of four with 100mM Tris-HCl pH 8.5 (final urea conc. = 2M)
2. Add 100 mM $CaCl_2$ to a final conc. of 1mM.
3. Add in trypsin 1µl (0.5µg/µl)
4. Incubate overnight at 37°C in the dark.

5. Add formic acid to 5% final concentration. Use only glass pipets for adding concentrated formic acid.

Solutions:

1M TCEP

for 1ml:

287mg

1ml MilliQ water

make a 1/10 dilution and store at –20°C in aliquots

500mM iodoacetamide

for 0.5ml:

46mg

500µl ddH_2O, make fresh

1M $CaCl_2$

for 100ml:

14.7g $CaCl_2 \cdot 2H_2O$

ddH_2O to 100ml