

LYS-C OR TRYPSIN IN-SOLUTION PROTEIN DIGEST PROTOCOL

Original protocol from the J. Yates Lab, Scripps Research Institute;
revised May '09 by Lori

If you have not already done so, please read our sample preparation tips.

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 80 μ l).
2. Add 100 mM TCEP (a reducing agent) to a final concentration of 5 mM.. Incubate at room temp. for 20 min (2.5 μ l if the total volume was 80 μ l).
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 (trypsin is recommended for mudPIT):

Trypsin Digest:

1. Dilute samples by a factor of four with 100mM Tris-HCl pH 8.5 (final urea conc. = 2M; if sample volume is a problem, dilute by only a factor of two to 4M urea)
2. Add 100 mM CaCl₂ to a final conc. of 1mM.
3. Add in trypsin 1 μ l (0.5 μ g/ μ l) Promega sequencing grade trypsin is recommended.
4. Incubate overnight at 37°C in the dark.

Lys-C Digest:

1. Add in Lys-C 1 μ l (0.1 μ g/ μ l), 1/100th total amount.
2. Incubate for 4 hr. at 37°C in the dark.

5. Add formic acid to 5% final conc.

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

500 μ l ddH₂O, make fresh

1M CaCl₂

for 100ml:

14.7g CaCl₂•2H₂O

ddH₂O to 100ml

filter sterilize

