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 80µl).
2. Add 100 mM TCEP (a reducing agent) to a final concentration of 5 mM. Incubate at room temp. for 20 min (2.5µl if the total volume was 80µl).
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 1µl (0.5µg/µl) Promega sequencing grade trypsin is recommended.
   4. Incubate overnight at 37°C in the dark.
   **Lys-C Digest:**
   1. Add in Lys-C 1µl (0.1µg/µl), 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
    - 500µl ddH₂O, make fresh
- **1M CaCl₂**
  - for 100ml:
    - 14.7g CaCl₂•2H₂O
    - ddH₂O to 100ml
    - filter sterilize