

## LYS-C OR TRYPSIN IN-SOLUTION PROTEIN DIGEST PROTOCOL

Reference: J. Yates Lab, Scripps Research Institute.

1. Bring solution up to 8M Urea and 100mM Tris-HCl pH 8.5 (40 $\mu$ l).
2. Add 0.12 $\mu$ l/40 $\mu$ l (5mM final conc.) total volume of 1M TCEP (a reducing agent). Incubate at room temp. for 20 min.
3. Add 0.88 $\mu$ l/40 $\mu$ l (10mM final conc.) total volume of 500mM iodoacetamide (make fresh daily, 0.046g/500 $\mu$ l ddH<sub>2</sub>O). Incubate at room temp. for 15 min. in the dark (covered with foil).

### Lys-C Digest:

1. Add in Lyse-C 1 $\mu$ l (0.1 $\mu$ g/ $\mu$ l), 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 (i.e. 120 $\mu$ l+40 $\mu$ l) with 100mM Tris-HCl pH 8.5 (final urea conc. = 2M)
2. Add 1M CaCl<sub>2</sub> to a final conc. of 1mM (i.e., 1.6 $\mu$ l/160 $\mu$ l)
3. Add in trypsin 1 $\mu$ l (0.5 $\mu$ g/ $\mu$ l)
4. Incubate overnight at 37°C in the dark.

Add formic acid to 5% final conc. Store at 20°C.

### Solutions:

1M TCEP

for 1ml:  
287mg  
1ml MilliQ water - filter  
store at -20°C in aliquots

500mM iodoacetamide

for 0.5ml:  
46g  
50 $\mu$ l ddH<sub>2</sub>O, make fresh

1M CaCl<sub>2</sub>

for 100ml:  
14.7g CaCl<sub>2</sub>o2H<sub>2</sub>O  
ddH<sub>2</sub>O to 100ml  
filter sterilize