

TRIPLE PROTEIN SOLUTION DIGEST PROTOCOL

Reference: J. Yates Lab, Scripps Research Institute. Protocol revised by Lori 6/2006.

Chymotrypsin may be substituted for either elastase or subtilysin; this is especially recommended when searching for methylation or acetylation. Use conditions like those for trypsin.

1. Bring solution up to 8M urea and 100mM Tris-HCl, pH 8.5 (ideal volume is about 100 μ l).
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 to a final concentration of 10mM (make fresh daily, 0.046g/500 μ l ddH₂O). Incubate at room temp. for 15 min. in the dark.
4. Divide the sample into three equal parts:

4A. Trypsin Digest:

1. Dilute samples by a factor of four (e.g., 120 μ l + 40 μ l) with 100mM Tris-HCl, pH 8.5 (final urea conc. = 2M)
2. Add 100 mM CaCl₂ to a final conc. of 1mM
3. Add in trypsin 1 μ l (0.5 μ g/ μ l)
4. Incubate overnight at 37°C in the dark.

4B. Elastase Digest:

1. Dilute samples by a factor of four (i.e. 120 μ l + 40 μ l) with 100mM Tris-HCl, pH 8.5 (final urea conc. = 2M)
2. Add in elastase 1 μ l (0.5 μ g/ μ l)
3. Incubate overnight at 37°C.

4C. Subtilysin Digest:

1. Add in subtilysin 1 μ l (0.36 μ g/ μ l)
2. Incubate overnight at 37°C.

5. Recombine 3 fractions into 1 sample. Add formic acid to 5% final conc.

Solutions:

1M TCEP	<u>for 1ml:</u> 287mg 1ml MilliQ water – filter store at –20°C in aliquots
500mM iodoacetamide	<u>for 0.5ml</u> 46g 50 μ l ddH ₂ O, make fresh

100 mM CaCl₂

for 100ml:
1.47g CaCl₂•2H₂O
ddH₂O to 100ml
filter sterilize