

Protocol for In-Solution Reduction/Alkylation (prior to loading the gel)

Reduction

1. Protein is lyophilized, precipitated or dissolved in 10 μL pH 7.5-8.5 buffer.
2. Resuspend protein in 20 μL 0.25% Zwittergent 3-16 (Calbiochem) in 200 mM ammonium bicarbonate.
3. Add 1 μL of 50 mM DTT (dithiothreitol) in water. Incubate 15 min at 60 $^{\circ}\text{C}$. Let cool. (Note: 50mM DTT = 0.77 g/mL)
- 4.

Alkylation

1. Add 5 μL of 22 mM iodoacetamide in water. Incubate 25 min at room temperature in the dark. (Note: 22 mM iodoacetamide= 0.004 g/mL)
2. Make a 1:10 dilution of the DTT solution from step 3 above and add 1.4 μL to the sample. Incubate 25 min at room temperature in the dark.