

BCA Protein Assay

Ann E. Hagerman © 2011

Reagents

CuSO₄ and Bicinchonic Acid Solution for the “Working Reagent”

Procedure

1. Thaw the samples and then place them on ice.
2. Turn on the computer and set up your protocol. Endpoint assay, 37 degrees with 30 min lag time, 562 nm absorbance, shake at “3” for 5 sec before reading
3. Set up a template
4. Pre-heat the plate reader to 37°C
5. Prepare standards using Sigma Protein Standard- 2 mg/mL

Dilute Sigma Protein (500 uL) with 500 uL of buffer = 1 mg/mL

Serial dilution

0.75 mg/mL	30 uL std with 10 uL IEF
0.5 mg/mL	20 uL std with 20 uL IEF
0.25 mg/mL	10 uL std with 30 uL IEF
0.1 mg/mL	10 uL std with 90 uL IEF
0.05 mg/mL	10 uL std with 190 uL IEF

6. Prepare the “Working Reagent” immediately before use by mixing CuSO₄ and the Bicinchonic Acid Solution in a 1:50 ratio (need 200 uL per sample or standard)
7. Dispense 10 uL of samples and standards into the wells according to the template.
8. Add 200 uL of Working Reagent into each well.
9. Start the Sigma Protein Protocol plate reading program.
10. Insert the plate into the plate reader
11. Begin the lag- time, a 30 minute incubation of the microplate at 37°C for 30 minutes.
12. Shake the plate in the plate reader.
13. Read the absorbance at 562 nm (an end-point absorbance reading).

MODIFIED 4-23-09