Protocol D: Preparation of BSA for quenching pretreatment

David Booth

Abstract

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Protocol

Make a 10% (w/v) solution of BSA

Step 1.

• Dissolve 2 g of bovine serum albumin, fraction V (Sigma 10735078001) in 20 ml of 50 mM HEPES-KOH pH 7.5, 200 mM NaCl, and 20% (v/v) glycerol

2. Sterile filter BSA solution through a 0.22 µm filter.

3. Prepare a small aliquot of a 100 fold dilution of BSA in 8 M Urea and measure the optical absorbance at 280 nm with a UV-Vis spectrophotometer. Determine the concentration of BSA where 1 absorbance unit is a concentration of 0.667 mg/ml BSA in a 1 cm pathlength.

4. Dilute BSA to a final concentration of 50 mg/ml with 50 mM HEPES-KOH pH 7.5, 200 mM NaCl, and 20% (v/v) glycerol that has been sterilly filtered through a 0.22 µm filter.

5. Make 1 ml aliquots and store at -20°C.

Sterile filter BSA solution through a 0.22 µm filter

Step 2.

Quantify BSA solution by UV-Vis spectrophotometry

Step 3.

• Prepare a small aliquot of a 100 fold dilution of BSA in 8 M Urea
• Measure the optical absorbance at 280 nm with a UV-Vis spectrophotometer
• Determine the concentration of BSA where 1 absorbance unit is a concentration of 0.667 mg/ml BSA in a 1 cm pathlength.

Dilute BSA to a final concentration of 50 mg/ml

Step 4.

• Dilute with 50 mM HEPES-KOH pH 7.5, 200 mM NaCl, and 20% (v/v) glycerol that has been sterilly filtered through a 0.22 µm filter

Store at -20°C

Step 5.