ARIA

Bradford Method for Lysis Verification

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Introduction

Bradford Method for protein quantification planned to be used for lysis verification. If L-Arabinose induced cultures did express the autolysis protein (Protein E), a high Absorbance 595nm measurement should be expected.

When coomassie dye binds protein in an acidic medium, an immediate shift in absorption maximum occurs from 465nm to 595nm with a concomitant color change from brown to blue. This event is taken advantage for protein quantification. Protein concentrations are estimated by reference to absorbances obtained for a series of standard protein dilutions (creation of a standard curve), which are assayed alongside the unknown samples.

Materials

Materials for Diluted Albumin (BSA) Standards

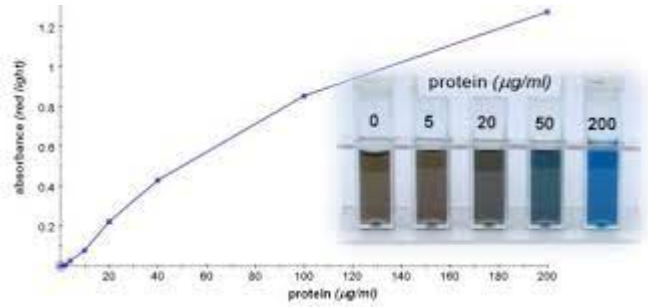
- Set of BSA dilutions. (2000μg/mL- 25μg/mL)
- Putative cell lysate
- Coomassie Reagent

Procedure

Bradford Method Assay

1. . Standard Test Tube Protocol (Working Range = 100-1500μg/mL)
2. Pipette 0.03mL (30μL) of each standard or unknown sample into appropriately labeled test tubes.
3. Add 1.5mL of the Coomassie Reagent to each tube and mix well.
4. Optional: For the most consistent results, incubate samples for 10 minutes at room temperature (RT).
5. With the spectrophotometer set to 595nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples.
6. Subtract the average 595nm measurement for the Blank replicates from the 595nm measurements of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 595nm measurement for each BSA standard vs. its concentration in μg/mL. Use the standard curve to determine the protein concentration of the lysate sample.

   As it can be seen in the figure, a curve should be built with the standard dilutions in order to determine the unknown quantity of protein in the cell lysate [1].



Bibliography

[1] Bradford Assay (Bradford Reagent) | Thermo Fisher Scientific - NL. (s. f.). Thermo Fisher Scientific. https://www.thermofisher.com/nl/en/home/life-science/protein-biology/protein-assays-analysis/protein-assays/bradford-assays.html