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1.2 Bradford assay

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[1]In-house protocol

1 Works for me This protocol is published without a DOI.

Eadewunm

ABSTRACT

Protein concentration estimation using Bradford assay

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

http://www.bio-rad.com/webroot/web/pdf/lsr/literature/LIT33.pdf

PROTOCOL CITATION

Elizabeth Fozo 2020. 1.2 Bradford assay. **protocols.io** https://protocols.io/view/1-2-bradford-assay-bqqqmvvw

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1 Prepare working reagent by diluting 1part Dye Reagent Concentrate with 4 parts distilled, deionized water. This diluted reagent may be used for approximately 2 weeks when kept at room temperature.

2 Prepare dilutions of a protein standard (Get from Becker lab- Sarah, they have aliquots for the diluted standards). The linear range of the assay for BSA is 0.2 to 0.9 mg/ml

3 Add 50ul of each standard into the cuvettes and add 2.5 ml of Bradford reagent (optimized from 100 ul + 5 ml)

4 Dilute your samples with 0.1% SDS in the cuvettes. For 1:10 dilution add 45ul of 0.1% SDS to the cuvette and add 5 ul of your sample (40 ul of 0.1% SDS and 10 ul sample for 1:5), mix properly. Add 2.5 mL of Bradford reagent to each tube and mix.

5 Incubate at room temperature for 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

6 Measure absorbance at 595 nm.

7 Plot the standard curve using standards and measure OD and results for the samples. Multiply the result with the dilution factor to get the concentration of your protein sample.

8 Use an excel sheet and calculate the amount of you sample to be mixed with the appropriate sample buffer to get a final concentration of 0.5 ug/ul.