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Bradford protein assay in 96-Well plate

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iGEM Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

Igem Dusseldorf

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Protocol status: Working
We use this protocol and it's working

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Keywords: well plate assay for quantification, µl of the diluted bradford, bradford protein, measurements protein concentration measurement, bsa stock solution to 200µg, diluted bradford, well plate assay, bradford reagent, bovine serum albumin, µl of solution buffer, solution buffer needed00200202018040401606060140808012010010010, reagent to each well, serum albumine standard, solution buffer, comparing measured absorbance, dilute the roti, amount of protein, bsa stock solution, measured absorbance, pipette 50µl of the standard, expected protein level, pipette 50 µl, od595 for each well, dilution, ml roti, pipette 50µl, concentration, ml of water, 200µg, ml in the same buffer, standard concentration

Abstract

Assay for quantification of protein by comparing measured Absorbance at 595 nm to bovine serum albumine standard

- Bovine serum albumin (concentrated >200 µg/ml)
- Roti-Quant 5x (ROTH)
- 96 Well Plate
- Plate Reader

Standard measurements
Dilute your BSA stock solution to 200µg/ml in the same buffer used for your solution of interest.
Dilute the Roti-Quant 5x reagent at a rate of 2:7,5. For 15 ml solution, this means 4 ml Roti-Quant added to 11 ml of Water.
Create 200 µl each of standard concentrations according to the following table:

	Concentration [µg/ml]	Volume of stock needed	Volume of solution buffer needed
	0	0	200
	20	20	180
	40	40	160
	60	60	140
	80	80	120
	100	100	100

Work in Triplicates. Pipette 50µl of the standards into the 96-Well plate
Add 200 µl of the diluted Bradford reagent to each well.
Incubate for 5 minutes at room temperature
Measure the OD_{595} for each well. Create a regression line from the averages of each concentrations' measurements
Protein concentration measurements
Dilute your solution of interest 1:20 or 1:40, depending on expected protein levels.
Work in triplicates and use blanks. Pipette 50 µl of each dilution into a well on the 96-Well plate.

When working with solutions containing pigments, prepare additional wells to which NO Bradford reagent will be added

Add 200 µl of the diluted Bradford reagent to each well. For the pigment controls, add 200 µl of solution buffer instead. Optimally, use a multi-pipette to reduce the time needed for adding the reagent.

Incubate for 5 minutes at room temperature

Measure the OD_{595} for each well.

When processing the data, correct the measurements for the blanks. When pigments are present in the solution, correct the OD_{595} by subtracting the OD_{595} of the pigment controls from the appropriate measurements.

Determine the amount of protein by putting the achieved OD_{595} of your solution of interest into the regression formula and multiplying by the dilution factor.