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Bradford Assay with RotiQuant (ROTH)

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1 Works for me dx.doi.org/10.17504/protocols.io.8gghttw

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ABSTRACT

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

MATERIALS TEXT

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

Standard measurements

1 Dilute your BSA stock solution to 200µg/ml in the same buffer used for your solution of interest.

2 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.

3 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

4 Work in Triplicates. Pipette 50µl of the standards into the 96-Well plate

5 Add 200 µl of the diluted Bradford reagent to each well.

6 Incubate for 5 minutes at room temperature

7 Measure the OD595 for each well. Create a regression line from the averages of each concentrations' measurements

Protein concentration measurements

8 Dilute your solution of interest 1:20 or 1:40, depending on expected protein levels.

9 Work in triplicates and use blanks. Pipette 50 µl of each dilution into a well on the 96-Well plate.

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

11 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.

12 Incubate for 5 minutes at room temperature

13 Measure the OD595 for each well.

14 When processing the data, correct the measurements for the blanks. When pigments are present in the solution, correct the OD595 by subtracting the OD595 of the pigment controls from the appropriate measurements.

15 Determine the amount of protein by putting the achieved OD595 of your solution of interest into the regression formula and multiplying by the dilution factor.