Oct 14, 2019

Bradford protein assay in 96-Well plate

iGEM Dusseldorf[1]

[1]Heinrich-Heine Universität Düsseldorf

1 Works for me dx.doi.org/10.17504/protocols.io.76xhrfn

iGEM Dusseldorf

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.

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

This is an open access protocol distributed under the terms of the **Creative Commons Attribution License** (https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited