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Chitin binding + Bradford assay

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Protocol status: Working

We use this protocol and it's working

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Abstract

This protocol can be used to measure the binding capacity of chitin binding proteins and visualizing this using a Bradford assay. The protocol has been adapted from:

F. Labroussaa, A. R. Zeilinger, and R. P. P. Almeida, "Blocking the Transmission of a Noncirculative Vector-Borne Plant Pathogenic Bacterium," *Mol. Plant-Microbe Interact.*, vol. 29, no. 7, pp. 535–544, Jul. 2016.

Chitin binding assay

- 1 First, prepare the following buffer:

Chitin buffer

KH ₂ PO ₄	2 mM	(0,272 g/L)
Na ₂ HPO ₄	8 mM	(1,424 g/L in case of Na ₂ HPO ₄ * 2 H ₂ O)
KCl	2 mM	(0,149 g/L)

Contents of the chitin buffer

Preparation of chitin solution. Make a chitin stock of 10 mg/ml in the chitin buffer.

- 2 In a 2 ml microfuge tube, add 50 ug of proteinin solution (chitin binding protein or a control, such as BSA).
- 3 Add500 ug of chitin from the chitin solution (50 ul in case of a 10 mg/ml stock solution).

Make sure the solution is being stirred while pipetting to make sure the solution is completely homogenized!

- 4 Adjust total volume to 500 ul.
- 5 Incubate themicrofuge tubeat room temperaturefor 1 hour while shaking (600 rpm on Eppendorf shaking block) to prevent chitin from precipitating.
- 6 After incubating, centrifuge for 3 minutes in a tabletop centrifuge at 13000 x g.

1h

Bradford assay

- 7 Make protein standards, with a known concentration (0 ug/ml, 1.25 ug/ml, 2.5 ug/ml, 5 ug/ml, 10 ug/ml, 15 ug/ml and 20 ug/ml).
- 8 Make dilutions for your sample (e.g. 5x and 20x).



- 9 Add Bradford Reagent (Sigma) 1:1 to your samples.
- 10 Let the samples incubate for 10 minutes at room temperature.
- 11 Transfer the solutions + reagent to 1 ml cuvettes.
- 12 First, measure the A595 (absorbance at 595 nm) for the sample containing 0 ug/ml protein. Use a photospectrometer. This will be the blank.
- 13 Measure the A595 for the rest of the protein standards
- 14 Use these to make a graph of A595 (y-axis) vs protein concentration (x-axis) and make a trendline + formula.
- 15 Measure the A595 of your protein samples. Make sure that the absorbance is within the value range of the standards. If not, measure the diluted sample(s) instead.
- 16 Calculate the protein concentration of your sample with the formula obtained from the trendline. Make sure to adjust your concentration in case you measured a diluted sample!
- 17 Plot your data

10m