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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.

Troubleshooting

Chitin binding assay

1 First, prepare the following buffer:

Chitin buffer

	KH2PO4	2 mM	(0,272 g/L)
	Na2HPO4	8 mM	(1,424 g/L in case of Na2HPO4 ∗ 2 H2O)
	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. 1h

6 After incubating, centrifuge for 3 minutes in a tabletop centrifuge at 13000 x g.

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. 10m

11 Transfer the solutions + reagent to 1 ml cuvettes.

12 First, measure the A595 (absorabance 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