Extraction and Lowry-Assay for determination of Synechocystis total protein

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Abstract

A quick Lowry.Assay for the extraction and determination of total protein from *Synechocystis.*

Photo credit: Miriam Dreesbach, Institute for Synthetic Microbiology, HHU Düsseldorf

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Before start

Remeber to prepare BSA standards to absolutely quantify your extracted total protein. A BSA range of 5 µg/ml to 100 µg/ml BSA in water or appropiate media showed good results.

Materials

Trichloroacetic acid View by P212121
Sodium Hydroxide S320 by Thermo Fisher Scientific
1kg Sodium Carbonate; Na2CO3 (anhydrous) RC-126 by G-Biosciences
Copper (II) sulfate pentahydrate CDB0063.SIZE.2.5Kg by Bio Basic Inc.
Folin & Ciocalteu's phenol reagent F9252 by Sigma Aldrich
Potassium sodium tartrate tetrahydrate S2377 by Sigma Aldrich

Protocol

Step 1.

Sample 1 ml of your *Synechocystis* culture in 2 ml Eppendorf tubes.

Step 2.

Add 110 µl of 100% trichloracetic acid and **incubate** the mixture on ice for 20 min.

DURATION
00:20:00 : Incubation on ice

Step 3.

Centrifuge your mixture for 10 min at 15,000 g at 4 °C.

DURATION
00:10:00 : Centrifugation

Step 4.

Discard the supernatant thoroughly and **place** the tubes **upright** for 10 min. **Tap** the upright tubes carefully until all liquid is removed.

Step 5.

Resuspend the pellet carefully in 500 µl of a 1 M NaOH solution. **Vortex** and **incubate** the samples over night (approx. 16 hours) at room temperature.

Step 6.

Prepare a Lowry solution by **mixing** the following reagents ***in the given order:***

- 500 µl K-Na-tartrate (2%)
- 500 µl Cu_2SO_4*5 H_2O (1%)
- 100 ml Na_2CO_3 (2%)

Scale the total volumes **down** to an appropriate amount. You will need 900 µl Lowry-Mix for each sample. Mix the Lowry-solution on the same day you use it and store it in the fridge in the meantime.

Step 7.

In a new 1.5 ml Eppendorf tube, **mix** 100 µl sample (in NaOH) and 900 µl Lowry-Mix.

Step 8.

Add 100 µl 50% Folin & Ciocalteu's phenol reagent (**diluted** in water). Immediately **incubate** ***in the dark*** for 45 min.

DURATION
00:45:00 : Incubation in the dark

Step 9.

Spin down your incubated solution at 14,000 g for 5 min to remove lipids and cell debris.

DURATION
00:05:00 : Centrifugation

Step 10.

Carefully **transfer** 1 ml of your Sample-Lowry-Folin-Mix into a plastic cuvette and measure extinction at 750 nm.

Step 11.

For absolute quantification, **prepare** a standard BSA concentration range from 5 to 100 µg/ml, and **follow** all steps above with your standard sample.

GOTO
BSA standard set -> go to step #1