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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

MATERIALS

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

BEFORE START INSTRUCTIONS

Remember 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 appropriate media showed good results.

- 1 **Sample** 1 ml of your *Synechocystis* culture in 2 ml Eppendorf tubes.
- 2 **Add** 110 µl of 100% trichloroacetic acid and **incubate** the mixture on ice for 20 min.
00:20:00 Incubation on ice
- 3 **Centrifuge** your mixture for 10 min at 15,000 g at 4 °C.
00:10:00 Centrifugation
- 4 **Discard** the supernatant thoroughly and **place** the tubes **upright** for 10 min. **Tap** the upright tubes carefully until all liquid is removed.
- 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.
- 6 **Prepare** a Lowry solution by **mixing** the following reagents *in the given order*:
 - 500 µl K-Na-tartrate (2%)
 - 500 µl Cu₂SO₄*5 H₂O (1%)
 - 100 ml Na₂CO₃ (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.
- 7 In a new 1.5 ml Eppendorf tube, **mix** 100 µl sample (in NaOH) and 900 µl Lowry-Mix.
- 8 **Add** 100 µl 50% Folin & Ciocalteu's phenol reagent (**diluted** in water). Immediately **incubate in the dark** for 45 min.

00:45:00 Incubation in the dark

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

00:05:00 Centrifugation

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

- 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.

go to step #1
undefined