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Extraction and Lowry-Assay for determination of *Synechocystis* total protein

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We use this protocol and it's working

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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

MATERIALS

- 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 **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**F9252
- Potassium sodium tartrate tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S2377

Troubleshooting

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.

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

2 **Add** 110 µl of 100% trichloracetic 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 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.

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 BSA standard set