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

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Protocol status: Working

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**

Before start

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 $\mu\text{g/ml}$, and **follow** all steps above with your standard sample.

 [go to step #1](#) BSA standard set