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# A simple, accurate, low cost method for starch quantification in green microalgae

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**Protocol status:** Working

**We use this protocol and it's working**

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- 1 Harvest microalgal cells from 10 mL culture using swing bucket centrifugation (2600 *g* for 3 min).
- 2 Transfer the cells to a 2-mL screw cap microtube. Spin briefly and get rid of the medium using a pippet. Freeze the cells immediately at -15 °C in an ice-crude sea salt mix.
- 3 Add 1 mL methanol/tetrahydrofuran mix (v/v =1/3) to the cell pellet to extract pigments. Shake the microtube occasionally. Extract the pigments for at least 30 min.
- 4 Centrifuge at 16,000 *g* at 4 °C for 10 min. Discard the supernatant. Repeat the pigment extraction until the pellet becomes white, then dry the pellet at 65 °C in an oven for one hour.
- 5 Wash the pellet out of the microtube repeatedly using 50 mM, pH 5 sodium phosphate buffer, 5 mL in total, and transfer to a 15-mL tube.
- 6 Autoclave the cell suspension at 134 °C for one hour to disintegrate the starch granules.
- 7 Mix well and transfer 1 mL of the autoclaved sample to a 2-mL microtube, add 0.5 g acid-washed glass beads and then use a mini-beadbeater (BioSpec Products, USA) to smash the cells and release the starch (three cycles at the highest speed).
- 8 Transfer 0.5 mL of the beaten sample to a microtube, add 0.5 mL sodium phosphate buffer (50 mM, pH 5) and 2 units (in 20 µL) of glucoamylase<sup>(1)</sup> for overnight digestion at 50 °C. Add the same amount of glucoamylase again the next morning for the second digestion in the same conditions for 7 hours.
- 9 After the double digestions, centrifuge the sample at 16,000 *g* at room temperature for 10 min and measure the glucose in the sample using the glucose assay protocol (the DNS method, see below).

## Glucose assay using the DNS method

- 10 Build the standard curve for glucose assay: Prepare 10 mM glucose solution in 50 mM sodium phosphate buffer, pH 5.0, and do 2-fold serial dilutions until 312 µM using the buffer.
- 11 Add 0.5 mL the glucose solution (or a sample) to 2 mL of the DNS reagent<sup>(2)</sup> in a test tube (need to include 2 blanks to set zero). Mix well then heat the mixture in boiling water for 5 min. Cool down in tap water and measure the OD<sub>540</sub> of the glucose standards and the samples.



- 12 Build the standard curve of OD<sub>540</sub> against glucose quantities. Use the regression equation to calculate the glucose quantities in the samples.

## Recipes and notes

- 13 (1) **Glucoamylase (TCI Chemicals, Tokyo, Japan; Cat. # M0035) prep.** : Take 100 units enzyme and dissolve it in 1 mL of 50 mM sodium phosphate buffer, pH 5 (17 mg of the enzyme powder contains about 100 units enzyme; the enzyme powder contains about 6000 units/g). This enzyme prep is good for 2 weeks if stored at 4 °C. Unit definition: amount of protein to cause degradation of 10 mg starch to glucose in 30 min (in specified conditions).
- 14 (2) **DNS reagent prep:** Dissolve 2 g NaOH in 70 mL H<sub>2</sub>O, add 1 g of 3,5-dinitrosalicylic acid (DNS), then add 30 g potassium sodium tartrate. When the all chemicals are dissolved (takes about 2 - 3 days using sonication occasionally), bring the final volume to 100 mL. The final concentrations of the chemicals: 0.5 M NaOH, 44 mM dinitrosalicylic acid, 1 M potassium sodium tartrate.
- 15 (3) This protocol is based on the manuscript "Optimization of starch quantification in green microalgae: a simple, accurate, low cost method using glucoamylase and dinitrosalicylic acid" submitted to PLOS ONE.