High-Throughput Beta-glucuronidase (GUS) assay for Phaeodactylum tricornutum

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ABSTRACT

A high-throughput method for measuring β-glucuronidase (GUS) activity in the diatom Phaeodactylum tricornutum. This protocol has been optimized for 250 µl volumes. For larger volumes see the following protocol dx.doi.org/10.17504/protocols.io.hefb3bn, which this protocol was based off of.

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KEYWORDS

Gus assay, diatoms, Phaeodactylum tricornutum, high-throughput

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BEFORE STARTING

**Phaeodactylum tricornutum** cultures were initially grown in 5 ml L1 + antibiotics in a 50-ml conical at 18°C until the cell concentration reached at least 1x10^6 cells ml^-1.

1. **Centrifuge**
   
   Transfer 250 µl of each *P. tricornutum* culture to a 96-well plate and centrifuge at 3000 x g for 10 min. Discard supernatant.

2. **Lyse**

   To lyse the cells, add 150 µl bacterial protein extraction reagent (B-PER, ThermoFisher) to each well and mix by pipetting.

3. **Centrifuge**

   Centrifuge plate for 10 min at 3000 x g. Transfer supernatants to a new 96-well plate, being careful not to disturb the cell debris.

4. **Extract**

   Transfer 50 µl of each lysate to a new plate and add 125 µl GUS extraction buffer + 1 mM MUG to each well. Incubate the plate for 1 h at 37°C.

   GUS extraction buffer = 50 mM NaPO_4 H_2 (pH 7), 0.1% Triton X-100, and 10 µM βME

5. **Stop Reaction**

   To stop the reaction, add 150 µl GUS stop buffer (0.2 M Na_2CO_3) to each well and mix by pipetting.

6. **Read fluorescence**

   Transfer 200 µl quenched reaction to an opaque 96-well plate.
7 Determine fluorescence using a plate reader. Settings: excitation- 360 nm; emission- 440 nm.

8 If fluorescence readings are too high to get a readout, dilute with additional stop buffer.

Normalization

9 Use remaining cell lysates to perform a BCA assay (or an equivalent assay) to normalize the GUS activity to total cell protein for each culture.