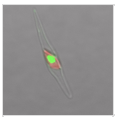

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High-Throughput Beta-glucuronidase (GUS) assay for Phaeodactylum tricornutum

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1 Works for me dx.doi.org/10.17504/protocols.io.bbexijfn

Protist Research to Optimize Tools in Genetics (PROT-G) JCVI West Protocols

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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.

MATERIALS

NAME	CATALOG #	VENDOR
MUG	MUG	Gold Biotechnology
Sodium carbonate	222321	Sigma – Aldrich
B-PER™ Bacterial Protein Extraction Reagent	78243	Thermo Fisher

MATERIALS TEXT

Flat bottom transparent and opaque 96-well plates
GUS extraction buffer- 50 mM $NaPO_4H_2$ (pH 7), 0.1% Triton X-100, and 10 µM βME + 1 mM 4-Methylumbelliferyl β-D-Glucuronide (MUG)
GUS stop buffer- 0.2 M Na_2CO_3
Plate reader
Swing bucket centrifuge with plate adapter

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}.

Centrifuge

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

Lyse

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

Centrifuge

3 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.

Extract

4 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 $NaPO_4H_2$ (pH 7), 0.1% Triton X-100, and 10 µM βME

Stop Reaction

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

6 Transfer 200 µl quenched reaction to an opaque 96-well plate.

Read fluorescence

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.