Beta-glucuronidase (GUS) assay (adapted for Phaeodactylum tricornutum)

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Abstract

A conventient extraction and measurement protocol for GUS activity from Phaeodactylum tricornutum.

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Protocol

Spin down cells

Step 1.

Centrifuge cells for 10 min at 3,000 x g. We typically use 4-5 mL cells grown to $1x10^6$ cells/mL.

Resuspend cells in extraction buffer

Step 2.

Pour off supernatant and resuspend cell pellets in 200 µL extraction buffer. Transfer to 1.5 mL microfuge tubes. Freeze cell suspension in liquid N_2 or dry ice/ethanol bath and either 1) store at -80C or 2) proceed with extraction and assay.

GUS extraction buffer:

50 mM sodium PO_4 buffer pH 7

10 µM beta-mercaptoethanol (BME)

0.1% Triton X-100

Perform freeze-thaw cycles

Step 3.

Do a total of three freeze-thaw cycles. Freeze on liquid N2 or with dry ice/ethanol and thaw by floating tubes in a rack in room temperature water. M

Centrifuge

Step 4.

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Spin down cell debris 5 min, 15,000 xg, 4 C. Transfer supernatant to a clean microfuge tube.

Prepare 96-well plates

Step 5.

Add 190 µL GUS+MUG buffer to each well in a 96 well plate. Do each P. tricornutum sample in duplicate or triplicate, so the total number of wells needed is equal to number of samples x 2 or 3. We standard clear, flat bottom 96 well plates for this step.

GUS+MUG buffer:

50 mL GUS extraction buffer + 17.6 mg MUG (4-Methylumbelliferyl-β-D-galactopyranoside)

Add samples to wells

Step 6.

Add 10 µL clarified sample (step 4) to wells, mix by gently tapping plate. Incubate 30-90 min at 37C.

Stop reaction

Step 7.

Prepare a stop buffer plate using a black 96-well flat bottom plate containing 180 µL stop buffer per well used in the reaction plate.

After incubation of reaction at 37 C for required time, transfer 20 µL of each reaction to the aliquotted stop buffer plate using a multichannel pipet. Mix by gentle tapping.

Stop Buffer: 0.2 M Na_2CO_3 (sodium carbonate)

Read reaction on fluorescent plate reader

Step 8.

Settings for fluorescence detection: excitation, 360 nm, emission, 440 nm

Freeze lysates for total protein quantification

Step 9.

Perform total protein quantification by BCA assay or equivalent. Normalize GUS activities to total protein.

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