Low Gel Temperature Agarose (LGTA) Media Version 2

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Abstract

Use for solid phase culture and chemical selection of *Pseudo-nitzschia spp* and other recalcitrant diatoms

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Guidelines

Reagents:

- 0.2 µm filtered seawater (FSW)
- L1 Medium Kit (ncma.bigelow.org)
- SeaPrep™ Agarose (Lonza Cat#: 50302, 25 gm)

Protocol

PREPARATION: 1% (w/v) LGTA
Step 1.

Add 100 mL FSW to autoclave compatible screw top bottle with stir bar.

PREPARATION: 1% (w/v) LGTA
Step 2.

To stirring FSW add:

- 1.00 gm SeaPrep agarose to stirring FSW, *avoid clumping*
- 0.10 mL L1 – NaH_2PO_4 stock (36.2 µM final, 1X L1 conc)
- 0.05 mL L1- $NaNO_3$ stock (441 µM final, 0.5X L1 conc)
- 0.20 mL L1 – Na_2SiO_3 stock (212 µM final, 2X L1 conc)
- 0.10 mL L1 – Trace Element stock (1X L1 conc)

NOTES
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Reagent sources listed under guidelines

PREPARATION: 1% (w/v) LGTA

Step 3.

Autoclave mixture to fully melt agarose.

PREPARATION: 1% (w/v) LGTA

Step 4.

Cool in water bath to 20° to 25°C with occasional stirring

PREPARATION: 1% (w/v) LGTA

Step 5.

Add 0.05 mL L1 – Vitamin stock (standard conc.), gently mix

NOTES

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NOTE: LGTA stocks can be held in molten form at ≥ 20°C or allowed to solidify at ≤ 15°C and remelted in microwave prior to cooling for use. *KEEP STERILE*

PLATING CULTURES:

Step 6.

Aliquot cooled, molten LGTA into sterile culture tubes (e.g. Falcon 2059).

- Volume should be sufficient to form thin layer (<5mm) in target plates or wells to enable in-gel imaging.
- *Recommend 0.2 mL per 24 well plate well, 2.5 mL for 30mm diameter petri plates, and 5 mL for 60 mm diameter plates.*

PLATING CULTURES:

Step 7.

Add selection reagents as needed.

PLATING CULTURES:

Step 8.

Add cell suspension ***into*** LGTA.

PLATING CULTURES:

Step 9.

Gently vortex to disperse cells.

PLATING CULTURES:

Step 10.

Transfer to culture plate avoiding introducing bubbles.

PLATING CULTURES:

Step 11.

Cool and incubate at 15°C to set agarose.

PLATING CULTURES:

Step 12.

Transfer to standard growth conditions as needed

- For species with high growth temperatures solidified LGTA can be transferred to appropriate incubation temperature after solidifying.
- For long term solid phase culture, the LGTA gel can be overlaid with L1 media.

PLATING CULTURES:

Step 13.

NOTE: Good gel strength is obtained at ≥ 0.75% (w/v) LGTA and optical clarity obtained at ≤ 2% LGTA).

We have had good luck with *Pseudo-nitzschia* growing in ≥0.75% to ≤1% LGTA.

Concentration of stock LGTA can be adjusted to accommodate larger culture volumes but a

1:1 (v/v) mixture of culture to LGTA should be considered the maximum mixing ratio for good gel formation.