Low Gel Temperature Agarose (LGTA) Media

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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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Protocol

REAGENTS:

Step 1.

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

PREPARATION: 1% (w/v) LGTA

Step 2.

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

b. 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)

c. Autoclave mixture to fully melt agarose.
d. Cool in water bath to 20° to 25°C with occasional stirring
e. Add 0.05 mL L1 – Vitamin stock (standard conc.), gently mix

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 3.

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a. 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.*

b. Add selection reagents as needed.

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

d. Gently vortex to disperse cells.

e. Transfer to culture plate avoiding introducing bubbles.

f. Cool and incubate at 15°C to set agarose.

g. 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.

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.

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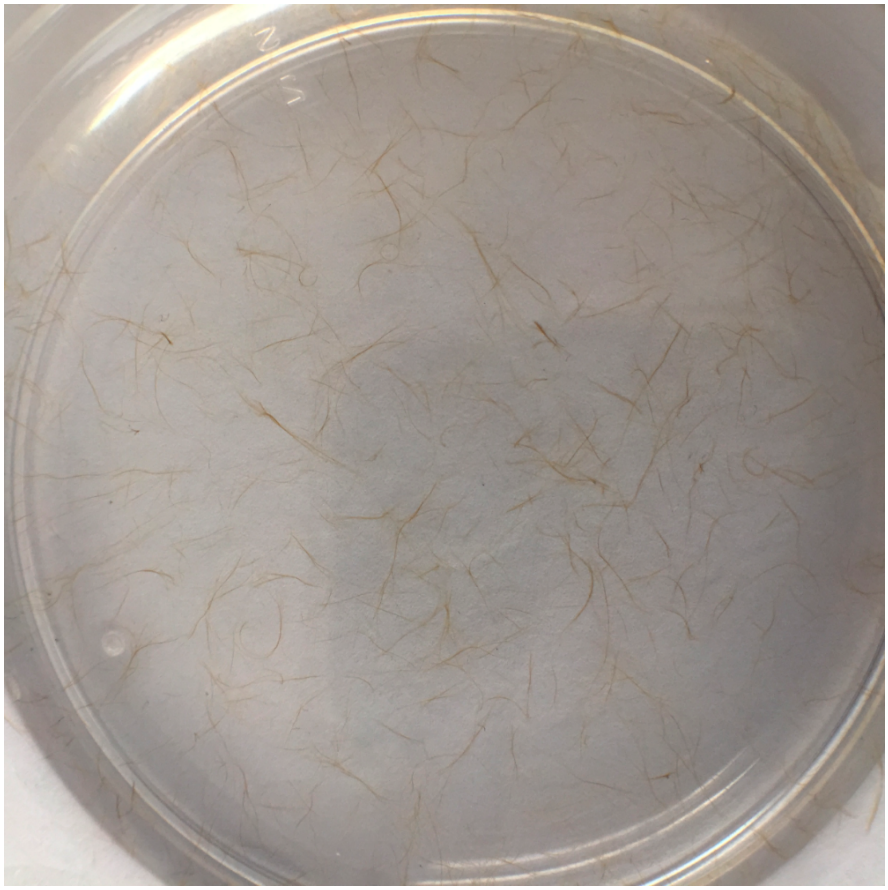

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