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Top agarose

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[1]Manual of Aquatic Viral Ecology

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Abstract

For use in "Isolation of cyanophages by plaque assyays"

Troubleshooting

Before start

Prepare 100 mL portions of 0.4 to 0.5% (w/v) of purified agar, agarose or low-melting point (LMP) agarose (i.e., Invitrogen #15517-022) in your media of choice. Although LMP agarose can be quite expensive, it is recommended for temperature sensitive samples and cells, since it solidifies at ca. 25°C. Purified agars, as well as low-melting point agars and agaroses are available for a range of lower temperatures (consult the following websites for more details: **www.sigmaaldrich.com** and **www.invitrogen.com**)

1 Autoclave or microwave sterilize on the day of the assay.

2 Dispense 2.5 to 3 mL into 13-×-100–mm glass disposable culture tubes (Fisher Scientific #1496127).

3 Transfer tubes to a water bath or dry heat block set at the appropriate temperature.

Note

(e.g., ca. 30 to 32°C for LMP agarose, ca. 40–42°C for purified agar or agarose)

4 Cover tubes with foil or cap, allow for temperature to equilibrate.

5 For each water sample, prepare triplicate tubes.

Note

Control tubes containing cells are only used to monitor lawn growth.

Note

For best results (smooth lump-free top agar), use freshly prepared top agar/agarose since repeated re-melting of solidified agar/agarose can give inferior results.