Base agar plates

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Abstract

For use in "Isolation of cyanophages by plaque assays"

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Guidelines

For example, purified agar or agarose (1% w/v) is added to your media of choice and autoclaved. This will provide a support base for the top agar/agarose overlay as well as nutrients for the host cells. For best results, use plates within 1 week of pouring.

Tip: plates can be fast-tracked: dry plates at 37°C; leave lids slightly ajar; monitor closely to prevent over drying.

Considerations: Depending on the composition of the media used, the addition of solidification agents (in particular the combination of high salinity seawater-based media and common agar such as Bacto Agar) can often result in the formation of precipitates when autoclaved together. These flocks can sometimes interfere with interpretation of the plaque assay. Moreover, impurities in common agar can negatively affect the growth of the host cells. Here are some suggestions on how to reduce the formation of these precipitates. Some testing may be required to determine the best combination to use for your particular situation.

a. Do not use common agar; rule of thumb the whiter the agar, the cleaner it is.
b. Use commercially available purified agar or agarose; or clean common agar using a washing procedure such as the one outlined in Waterbury and Willey (1989).
c. Reduce the salinity of seawater media with purified water; e.g., to 20-25 psu.
d. Add purified agar or agarose to autoclaved media aseptically and then melt the agar/agarose in the microwave (bring to a short boil 2-3 times to completely dissolve the agar/agarose).
e. For cells that will grow in artificial media, prepare media and gelling agent at 2x concentration and autoclave separately. When cooled to ca. 60°C, gently mix the gelling agent into the media and dispense immediately.
f. In the case of artificial media, add agar/agarose to filter-sterilized media and melt the gelling agent in the microwave

Protocol

Prepare base plates

Step 1.
Add 5 g purified agar or agarose to 500 mL culture media in a 1-L Erlenmeyer or media bottle.

Prepare base plates

Step 2.

Gently stir to disperse the agar/agarose.

Prepare base plates

Step 3.

Autoclave for 20 to 25 min to sterilize.

DURATION

00:25:00

Prepare base plates

Step 4.

When cooled to about 60°C, dispense 15 to 20 mL per plate.

NOTES

Amy Chan 02 Feb 2016

the suggested volume is suitable for 15 x 100 mm diameter petri plates; adjust accordingly for smaller or larger plates

Prepare base plates

Step 5.

To reduce condensation forming on the insides of the lids, leave lids slightly ajar to allow escape of steam or stack the plates immediately after pouring.

Prepare base plates

Step 6.

Invert plates once the agar has solidified to prevent condensation from dripping onto the surface of the agar.

Prepare base plates

Step 7.

Plates can be used about 12 h after pouring if the agar surface is not wet; a longer time is needed if conditions are humid.

DURATION

12:00:00

NOTES

Amy Chan 30 Sep 2015

If the surface of the bottom agar is too moist, the top agar/agarose will not stick to the bottom plate and will slide off when the plate is inverted.

Warnings

If the surface of the bottom agar is too moist, the top agar/agarose will not stick to the bottom plate and will slide off when the plate is inverted.