MEDIA CONTAINING AGAR OR AGAROSE

Prepare liquid media according to the recipes given above. Just before autoclaving, add one of the following:

bacto-agar	(for plates) 15 g/liter
bacto-agar	(for top agar) 7 g/liter
agarose	(for plates) 15 g/liter
agarose	(for top agarose) 7 g/liter

Sterilize by autoclaving for 20 minutes at 15 lb/sq. in. on liquid cycle. When the medium is removed from the autoclave, swirl it gently to distribute the melted agar or agarose evenly throughout the solution. *Be careful! The fluid may be superheated and may boil over when swirled.* Allow the medium to cool to 50°C before adding thermolabile substances (e.g., antibiotics). To avoid producing air bubbles, mix the medium by swirling. Plates can then be poured directly from the flask; allow about 30–35 ml of medium per 90-mm plate. To remove bubbles from medium in the plate, flame the surface of the medium with a bunsen burner before the agar or agarose hardens. Set up a color code (e.g., two red stripes for LB-ampicillin plates; one black stripe for LB plates, etc.) and mark the edges of the plates with the appropriate colored markers.

When the medium has hardened completely, invert the plates and store them at 4°C until needed. The plates should be removed from storage 1–2 hours before they are used. If the plates are fresh, they will "sweat" when incubated at 37°C. This allows bacterial colonies or bacteriophage plaques to spread across the surfaces of the plates and increases the chances of cross-contamination. This problem can be avoided by wiping off any condensation from the lids of the plates and then incubating the plates for several hours at 37°C in an inverted position before they are used. Alternatively, the liquid can be removed by shaking the lid with a single, quick motion. To minimize the possibility of contamination, hold the open plate in an inverted position while removing the liquid from the lid.