AgBiotech in Action
Are You Sterile?

Standard Operating Procedure

Agar Preparation without an Autoclave

Laboratory: Biotechnology
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General: This procedure is for the aseptic preparation of agar plates. The powdered agar medium bottle will have directions for preparing 1 L of agar. The flask that you are using needs to be at least twice the size of the amount of agar that you are preparing. The protocol listed below is for the preparation of 500 ml of agar in a 1000 ml Erlenmeyer flask.

For additional sterilization methods a good resource is located at www.umsl.edu/~microbes/sterilizing.pdf. Flinn Scientific has a video with another method at their website. Log onto flinnsci.com. click on Teacher Resources across the top. Click on Teacher Resource Videos at the bottom of the page. Click on Minute Videos. Click on Biology Videos. Select "Sterilizing without an Autoclave."

Safety: PPE—eye protection, hot gloves, aprons

Materials

powdered agar medium
distilled water
cotton plug
graduated cylinder
Petri dishes
1 L Erlenmeyer flask

Tools and equipment: stir rod, hot plate, autoclave, balance

Procedure

1. The powdered agar medium bottle will have directions for preparing 1 L of agar. Divide the number of grams of powdered agar needed by 2 for the preparation of 500 ml. Measure out the powdered agar medium using a balance.
2. Measure 500 ml of distilled water into a graduated cylinder.
3. Pour the distilled water into the 1 L flask.
4. Pour 11.5g of the agar into the flask.
5. Swirl the flask to mix and add a magnetic stir bar.
6. Cover the flask with foil and heat with repeated stirring on the hot plate on high until the liquid is just boiling. Agar will boil over quickly.
7. Simmer the liquid for 30 minutes on low heat.
8. Allow the agar medium to cool to about 50^{o} C before pouring into agar plates.
9. Pour the agar into the bottom of the Petri dish until 1/3 to 1/2 full.
10. Place the lid on the petri dish.
11. Agar plates sterilized in this manner are good for one to two days.
12. Option: if you only need to make small batch of 4 plates, use 125ml of distilled water in step 2 and pour into a 250ml flask. Then for step 4, weigh out 5.75g of agar and add to flask.