

SOP: M010

Preparation of Middlebrook 7H11-Dextrose agar protocol

Materials and Reagents:

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Middlebrook 7H11 agar (VWR 90004-942)
6. Dextrose (VWR 90000-908)
7. Glycerol (VWR IC800689)
8. Graduated cylinder, 1 liter
9. Autoclave
10. Water bath, 55°C
11. Serological pipet, 50 ml, sterile
12. Electric pipettor
13. Sterile plates, 15 x 150 mm or 15 x 100 mm.
14. Serological pipet, 10 ml, sterile
15. Sharpie marker
16. Ziploc bag, one gallon

Protocol:

1. ____ Pour 700 ml of Milli-Q water into a 1 liter beaker.
2. ____ Add magnetic stir bar to beaker and place on magnetic stir plate.
3. ____ Add 21.0 g of Middlebrook 7H11 dehydrated agar.
4. ____ Add 2.0 g of dextrose to make a final concentration of 2% (note 1)
4. ____ Make sure all components are completely in solution.
5. ____ Add 5 ml of glycerol.
6. ____ Make sure the glycerol is fully dispersed.
7. ____ Pour medium into 1 liter graduated cylinder.
8. ____ Bring volume to 900 ml with Milli-Q water.
9. ____ Transfer/aliquot to desired container(s) (note 2).
10. ____ Autoclave on liquid cycle (slow exhaust) at 121°C for 15 minutes.
11. ____ Place sterile medium in 55°C water bath for 30 minutes (note 3).
12. ____ Pour agar into plates (note 4).
13. ____ Remove any bubbles on plates by pipetting with a 10 ml pipet and electric pipettor.
14. ____ Allow plates to cool and solidify.
15. ____ Label plates and store at 4°C in a Ziploc bag.

Notes:

1. This is the amount of dextrose present when OADC is added to the medium at a final concentration of 10%. Therefore, OADC does not need to be added.
2. It is best to make up the desired amount of agar in each container/volume desired instead of making aliquots from a 900 ml stock; otherwise, the solution needs to be brought to a boil to completely re-suspend the agar prior to making aliquots. If the agar is not boiled, then it will be unevenly dispersed between containers and the plates will not solidify correctly.
3. Allows the agar solution to cool to a temperature that allows for handling without solidifying the agar.
4. One batch of 7H11 agar with dextrose will make approximate six 15 x 150 mm plates or thirteen 15 x 100 mm plates. Plates should be poured thickly to ensure they do not completely dry out when used for culturing of *M. tuberculosis*.

Reference:

Diffco manual, 10th edition. 1984 Difco Lab, Inc. Detroit, MI 48232.