

SOP: M010.1**Date modified 12/19/19 AS****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)
5. _____ Make sure all components are completely in solution.
6. _____ Add 5 ml of glycerol.
7. _____ Make sure the glycerol is fully dispersed.
8. _____ Pour medium into 1 liter graduated cylinder.
9. _____ Bring volume to 900 ml with Milli-Q water.
10. _____ Transfer/aliquot to desired container(s) (note 2).
11. _____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.
12. _____ Place sterile medium in 55°C water bath for 30 minutes (note 3).
13. _____ Turn on and clean BioSafety Cabinet (note 4).
14. _____ Inside the BSC, pour agar into plates (note 5).
15. _____ Remove any bubbles on plates by pipetting with a 10 ml pipet and electric pipettor.
16. _____ Allow plates to cool and solidify.
17. _____ 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. See SOP SP041.
5. 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.