

**SOP: M009**

**Preparation of Middlebrook 7H11 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. Glycerol (VWR IC800689)
7. Graduated cylinder, 1 liter
8. Autoclave
9. Water bath, 55°C
10. OADC solution (VWR 90000-614) (note 1)
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. \_\_\_\_ 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. \_\_\_\_ Add 100 ml of sterile OADC solution to agar solution.
13. \_\_\_\_ Pour agar into plates (note 4).
14. \_\_\_\_ Remove any bubbles on plates by pipetting with a 10 ml pipet and electric pipettor.
15. \_\_\_\_ Allow plates to cool and solidify.
16. \_\_\_\_ Label plates and store at 4°C in a Ziploc bag.

**Notes:**

1. OADC is packaged in 500 ml bottles; however, this is divided into 100 ml aliquots by workstudies, and tested for sterility prior to storage in a common location.
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. If the OADC is added to the sterile medium while the medium is too hot, the components of the OADC will degrade.
4. One liter of 7H11 agar with 10% OADC will make approximate seven 15 x 150 mm plates or fifteen 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:**

Difco manual, 10<sup>th</sup> edition. 1984 Difco Lab, Inc. Detroit, MI 48232.