

SOP: M018.2

Updated 01/11/23 DRM

Preparation of Middlebrook 7H11 agar + 40mM Sodium pyruvate 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. 8 Autoclave
9. Water bath, 55°C
10. OADC solution (VWR 90000-614)
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
17. Sodium pyruvate

Protocol:

- 1.____ Pour 900 ml of Milli-Q water into a 1liter 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.____ Add 4.4g Sodium pyruvate.
- 8.____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.
- 9.____ Place sterile medium in 55°C water bath for 30 minutes (Note 1).
- 10.____ Clean and set up the Biosafety cabinet (Note 2).
- 11.____ Add 100 ml of sterile OADC solution to agar solution (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. If you are immediately making the agar, let it cool until it is safe to touch with gloves on and use a 50mL pipette to remix the agar inside (instead of placing in a water bath).
2. See SOP SP041.
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:

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