SOP: M026

Preparation of Middlebrook 7H11 Agar protocol for Lys and Pan Auxotroph

Materials and Reagents:

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Middlebrook 7H11 Agar (VWR 90003-876)
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. Biosafety cabinet
12. Serological pipet, 50 ml, sterile
13. Electric pipettor
14. (3X) 250ml Filter Units (0.22 µm, PES membrane) (VWR 167-0045)
15. **Glycerol (50% stock solution) (note 5)**
16. **Casamino Acids (20% stock solution) (note 6)**
17. **Pantothenate (48 mg/ml) (note 7)**
18. **Leucine (50 mg/ml) (note 8)**

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 g of Middlebrook 7H11 dehydrated broth.

4._____ Add 10 ml of 50% glycerol.

5._____ Make sure all components are completely in solution.

6._____ Bring the volume to 900 ml with Milli-Q water.

7._____ Pour into 1L Glass media bottle (orange cap, pyrex).

8._____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.

9._____ Aseptically add 100 ml of sterile OADC solution to medium (note 1-4).

10._____ Aseptically add 10 ml of 20% Casaminoacids.

11._____ Aseptically add 1 ml of Pantothenate (48 mg/ml).

12._____ Aseptically add 1 ml of L-leucine (50 mg/ml).

13._____ If using medium for a KO organism, add antibiotic before pouring agar into plates.

14._____ Make sure all components are completely in solution (note 9).

15._____ Pour agar into plates (note 10).

16._____ Allow plates to cool and solidify, then wrap with parafilm.

17._____ 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. If the OADC is added to the sterile medium while the medium is too hot, the components of the OADC will degrade. Add the OADC when medium is around 60-65°C. This must be done before medium solidifies.
3. See SOP SP041 for sterile use of the biosafety hood. After autoclaving, all components should be added in biosafety hood to avoid contamination.
4. Do not return any remaining OADC to common supply area; keep as own personal stock.
5. 50% Glycerol – add 25 ml of glycerol to 25 ml of milliQ water.
6. 20% Casaminoacids – 20 g/100 ml (10g/50 ml milliQ water). Sterile filter before adding to medium.
7. Pantothenate – 2.4 g D-Pantothenic acid in 50 mL of milliQ water. Sterile filter with 0.22 µm filter before adding to medium.
8. L-Leucine (2 g/30 ml milliQ water. Add 3.84 ml of **35% HCl** (35ml/100ml H20, 3.5ml/10ml H20). Sterile filter before adding to medium and store remainder of solution at 4°C.
9. Make sure to add all components before medium solidifies.
10. One batch of Agar will make approximate nine 15 x 150 mm plates or twenty 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.