

SOP: M006

Preparation of Middlebrook 7H9 broth + 0.05% Tween 80 protocol

Materials and Reagents:

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Middlebrook 7H9 broth (VWR 90003-876)
6. Glycerol (VWR IC800689)
7. Tween 80 (Fisher T164-500), 20% solution, sterile
8. Graduated cylinder, 1 liter
9. Autoclave
10. Water bath, 55°C
11. OADC solution (VWR 90000-614) (note 1)
12. Biosafety cabinet
13. Serological pipet, 50 ml, sterile
14. Electric pipettor

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 4.7 g of Middlebrook 7H9 dehydrated broth.
4. ____ Make sure all components are completely in solution.
5. ____ Add 2 ml of glycerol.
6. ____ Make sure the glycerol is fully dispersed.
7. ____ Add 2.5 ml of 20% Tween solution to make a final Tween concentration of 0.05%.
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).
11. ____ Autoclave on liquid cycle (slow exhaust) at 121°C for 15 minutes.
12. ____ Place sterile medium in 55°C water bath for 30 minutes (note 2).
13. ____ Turn on and clean BioSafety Cabinet (note 3).
14. ____ Place sterile pipet, OADC and sterile medium inside BSC.
15. ____ Aseptically add 100 ml of sterile OADC solution to medium (note 4).
16. ____ Remove items from BSC and clean inside of cabinet (note 3).

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. It is also acceptable to allow the medium to cool to room temperature prior to the addition of OADC, as the OADC may be added anytime prior to use.
3. See SOP SP041.
4. Do not return any remaining OADC to common supply area; keep as own personal stock.

Reference:

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