

SOP: M008

Preparation of Middlebrook 7H9-Dextrose 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. Dextrose (VWR 90000-908)
7. Glycerol (VWR IC800689)
8. Tween 80 (Fisher T164-500), 20% solution, sterile
9. Graduated cylinder, 1 liter
10. Autoclave

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. ____ Add 2.0 g of Dextrose to make a final concentration of 2% (note 1).
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.

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.

Reference:

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