

SOP: M017.2**Updated 01/13/22 DRM****Preparation of Middlebrook 7H9 broth + 0.05% Tween 80 + 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 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)
12. Biosafety cabinet
13. Serological pipet, 50 ml, sterile
14. Electric pipettor
15. 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 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.____ Add 4.4g Sodium pyruvate.
- 9.____ Transfer/aliquot to desired container(s).
- 10.____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.
- 11.____ Let cool on counter.
- 12.____ Turn on and clean BioSafety Cabinet (note 1).
- 13.____ Place sterile pipet, OADC and sterile medium inside BSC.
- 14.____ Aseptically add 100 ml of sterile OADC solution to medium (note 2).
- 15.____ Remove items from BSC and clean inside of cabinet.

Notes:

1. See SOP SP041.
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 any time prior to use.

Reference:

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