

**SOP: M006.2**

**Updated 01/11/23 DRM**

**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)
12. Biosafety cabinet
13. Serological pipet, 50 ml, sterile
14. Electric pipettor

**Protocol:**

1. \_\_\_\_\_ Pour 895.5 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. \_\_\_\_\_ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.
9. \_\_\_\_\_ Turn on and clean BioSafety Cabinet (Note 1).
10. \_\_\_\_\_ Place sterile pipet, OADC and sterile medium inside BSC.
11. \_\_\_\_\_ Aseptically add 100 ml of sterile OADC solution to medium (Note 2).
12. \_\_\_\_\_ 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:**

Diffco manual, 10<sup>th</sup> edition. 1984 Difco Lab, Inc. Detroit, MI 48232.