

**SOP: M007**

**Preparation of Middlebrook 7H9-Dextrose broth 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. Graduated cylinder, 1 liter
9. 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. \_\_\_\_ Pour medium into 1 liter graduated cylinder.
8. \_\_\_\_ Bring volume to 900 ml with Milli-Q water.
9. \_\_\_\_ Transfer/aliquot to desired container(s).
10. \_\_\_\_ 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, 10<sup>th</sup> edition. 1984 Difco Lab, Inc. Detroit, MI 48232.