SOP: M016.2
Updated 01/11/23 DRM

Preparation of Middlebrook 7H9 broth + 40 mM 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. Graduated cylinder, 1 liter
8. Autoclave
9. Water bath, 55°C
10. OADC solution (VWR 90000-614)
11. Biosafety cabinet
12. Serological pipet, 50 ml, sterile
13. Electric pipettor
14. 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 4.4g Sodium pyruvate.

8._____ Transfer/aliquot to desired container(s).

9._____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.

10._____ Let cool on counter.

11._____ Turn on and clean BioSafety Cabinet (note 1).

12._____ Place sterile pipet, OADC and sterile medium inside BSC.

13._____ Aseptically add 100 ml of sterile OADC solution to medium (note 2).

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