

## SOP: M025

### Preparation of Middlebrook 7H9 broth protocol for Lys and Pan Auxotroph

#### 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. Water bath, 55°C
9. OADC solution (VWR 90000-614) (note 1)
10. Biosafety cabinet
11. Serological pipet, 50 ml, sterile
12. Electric pipettor
13. 1 L Filter Unit (0.22 µm, PES membrane) (VWR 167-0045)
14. **Glycerol (50% stock solution) (note 3)**
15. **Tyloxapol (5% stock solution) (note 4)**
16. **Casamino Acids (20% stock solution) (note 5)**
17. **Pantothenate (48 mg/ml) (note 6)**
18. **L-leucine (50 mg/ml) (note 7)**

#### 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 10 ml of 50% glycerol.
5. \_\_\_\_\_ Bring volume to 900 ml and make sure all components are completely in solution.
6. \_\_\_\_\_ Pour into 1L Glass media bottle (orange cap, pyrex).
7. \_\_\_\_\_ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes.
8. \_\_\_\_\_ Wait for solution to cool to about 60-65°C and aseptically add 100 ml of sterile OADC solution to medium (note 2).
9. \_\_\_\_\_ Aseptically add 10 ml of 5% Tyloxapol.
10. \_\_\_\_\_ Aseptically add 10 ml of 20% Casaminoacids.
11. \_\_\_\_\_ Aseptically add 1 ml of Pantothenate (48 mg/ml)
12. \_\_\_\_\_ Aseptically add 1 ml of L-leucine (50 mg/ml)
13. \_\_\_\_\_ Make sure all components are fully dispersed.
14. \_\_\_\_\_ If using medium for growth of a KO organism, aseptically add 1 ml of antibiotic to 1 L of medium.

#### Notes:

1. OADC is packaged in 500 ml bottles; however, this is divided into 100 ml aliquots by workstudies, and tested for sterility prior to storage in a common location.

2. Do not return any remaining OADC to common supply area; keep as own personal stock.
3. 50% Glycerol – add 25 ml of glycerol to 25 ml of milliQ water.
4. 5% Tyloxapol – 5 g/100ml (2.5 g/50 ml milliQ water)
5. 20% Casaminoacids – 20 g/100 ml (10g/50 ml milliQ water). Sterile filter with 0.22  $\mu$ m filter.
6. Pantothenate – 2.4 g D-Pantothenic acid in 50 mL of milliQ water. Sterile filter with 0.22  $\mu$ m filter.
7. L-Leucine (2 g/30 ml milliQ water. Add 3.84 ml of **35% HCl** (35ml/100ml H<sub>2</sub>O, 3.5ml/10ml H<sub>2</sub>O). Sterile filter and store at 4°C. (To make 35% HCl add 3.584 ml of 12M HCl to 0.256 ml of H<sub>2</sub>O).

**Reference:**

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