SOP: M002

Preparation of GAS medium + 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. BactoCasitone (BD Science BD225930)
6. Ferric ammonium citrate (Sigma F-5879)
7. Potassium phosphate, dibasic anhydrous (VWR MK709208) (note 1)
8. Citric acid, anhydrous (VWR JT0122-1)
9. L-Alanine (Sigma A-7627)
10. Magnesium chloride, heptahydride (VWR MK595804)
11. Potassium sulfate (VWR MK714004)
12. Ammonium chloride (VWR MK338412)
13. Tween 80 (Fisher T164-500), 20% solution, sterile
14. Sodium hydroxide, 10 M
15. Glycerol (VWR IC800689)
16. Graduated cylinder, 1 liter
17. Autoclave

Protocol:

1. _____ Pour 800 ml of Milli-Q water into a 1 liter beaker.

2. _____ Add magnetic stir bar to beaker and place on stir plate.

3. _____ Add 0.3 g of BactoCasitone.

4. _____ Add 0.05 g of ferric ammonium citrate.

5. _____ Add 4.0 g of potassium phosphate, dibasic anhydrous (note 1).

6. _____ Add 2.0 g of citric acid.

7. _____ Add 1.0 g of L-alanine.

8. _____ Add 1.2 g of magnesium chloride.

9. _____ Add 0.6 g of potassium sulfate.

10. _____ Add 2.0 g of ammonium chloride.

11. _____ Make sure all components are completely in solution.

12. _____ Add 1.8 ml of 10 M sodium hydroxide.

13. _____ Make sure the sodium hydroxide is completely in solution.

14. _____ Add 10.0 ml of glycerol.

15. _____ Make sure the glycerol is fully dispersed.

16. _____ Add 2.5 ml of 20% Tween solution to make a final Tween concentration of 0.05%.

17. _____ Measure the pH, and adjust to 6.6.

18. _____ Pour medium into 1 liter graduated cylinder.

19. _____ Bring volume to 1 liter with Milli-Q water.

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

21. _____ Autoclave on liquid cycle (slow exhaust) at 121^{o}C for 15 minutes (note 2).

Notes:
1. **MUST** use Mallinckrodt K_2HPO_4; other brands will salt out during sterilization.
2. The medium will appear cloudy immediately after sterilization, but will clear upon cooling.

Reference:
Takayama, K., H. K. Schnoes, E. L. Armstrong, and R. W. Boyle. 1975. Site of inhibitory action of isoniazid in the synthesis of mycolic acids in Mycobacterium tuberculosis. J. Lipid Res. 16: 308-317.