

SOP: M032

Written: 1/13/23 DRM

Preparation of GAS medium + 0.05% Tween 80 protocol + 20% glycerol

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)
8. Citric acid, anhydrous (VWR JT0122-1)
9. L-Alanine (Sigma A-7627)
10. Magnesium chloride, hexahydrate (VWR MFCD00149781)
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 785mL 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.
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 200 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. _____ Autoclave on liquid cycle (slow exhaust) at 121°C for 45 minutes (Note 1).

Notes:

1. 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.