

SOP: M004

Preparation of modified Sauton's + 0.05% Tween 80 medium protocol

Materials and Reagents:

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Potassium phosphate, monobasic (VWR MK710012)
6. Magnesium sulfate, anhydrous (VWR MK607012)
7. L-Asparagine (Sigma A-7627)
8. Ferric ammonium citrate (Sigma F-5879)
9. Citric acid, anhydrous (VWR JT0122-1)
10. Glycerol (VWR IC800689)
11. Zinc sulfate, heptahydrate, (Sigma Z-0251) 1% solution, sterile
12. Tween 80 (Fisher T164-500), 20% solution, sterile
13. Sodium hydroxide, 10 M
14. Graduated cylinder, 1 liter
15. 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 magnetic stir plate.
3. ____ Add 0.5 g of potassium phosphate, monobasic.
4. ____ Add 0.5 g of magnesium sulfate.
5. ____ Add 4.0 g of L-asparagine.
6. ____ Add 0.05 g of ferric ammonium citrate.
7. ____ Add 2.0 g of citric acid.
8. ____ Make sure all components are completely in solution.
9. ____ Add 47.6 ml of glycerol.
10. ____ Make sure glycerol is completely dispersed.
11. ____ Add 0.1 ml of zinc sulfate solution.
12. ____ Make sure zinc sulfate solution is completely dispersed.
13. ____ Add 2.5 ml of 20% Tween solution to make a final Tween concentration of 0.05%.
14. ____ Measure the pH, and adjust to between 6.8 and 7.2 using 10 M sodium hydroxide.
15. ____ Pour medium into a 1 liter graduated cylinder.
16. ____ Bring volume to 1 liter with Milli-Q water.
17. ____ Transfer to desired container(s).

18. _____ Autoclave on liquid cycle (slow exhaust) at 121°C for 15 minutes.