

**SOP: M003**

**Preparation of modified Sauton's 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. Sodium hydroxide, 10 M
13. Graduated cylinder, 1 liter
14. 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. \_\_\_\_ Measure the pH, and adjust to between 6.8 and 7.2 using 10 M sodium hydroxide.
14. \_\_\_\_ Pour medium into a 1 liter graduated cylinder.
15. \_\_\_\_ Bring volume to 1 liter with Milli-Q water.
16. \_\_\_\_ Transfer/aliquot to desired container(s).
17. \_\_\_\_ Autoclave on liquid cycle (slow exhaust) at 121°C for 15 minutes.