

# Folate Microbiologic Assay Training Poster

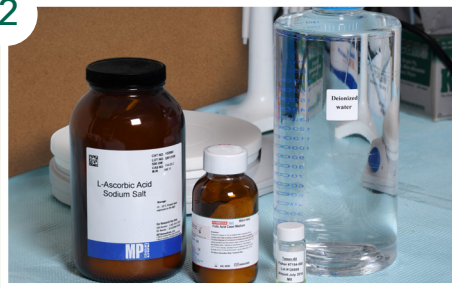
## Part 1: Prepare Reagents

1



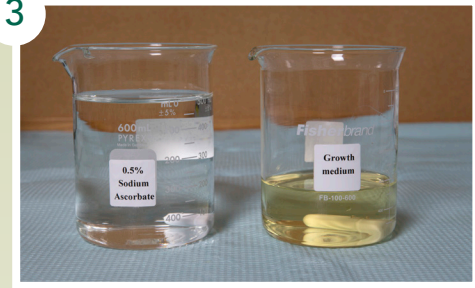
The frozen assay kit contains (a) microorganism (*L. rhamnosus*); (b) calibrator stock solution (5-methyltetrahydrofolate); (c) 2 levels of quality control materials; and (d) three reagents (ascorbic acid, chloramphenicol, and manganese sulfate) required for the growth medium preparation.

2



Not provided with the kit are the following items: growth medium, Tween-80, sodium ascorbate, and deionized water.

3



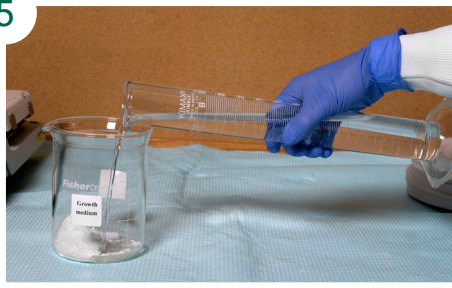
Two reagents need to be prepared freshly for each run: 0.5% sodium ascorbate and growth medium containing the microorganism.

4



Sodium ascorbate solution preparation: To prepare a 0.5% solution, add 2.5 g of sodium ascorbate to a 500-mL beaker. Add 500 mL of deionized water, mix well. Cover the beaker with foil.

5



Medium preparation – Step 1: To prepare 200 mL of growth medium, add 14.1 g of Folic Acid Casei Medium to a 500-mL beaker. Add 200 mL of deionized water, mix well. Cover the beaker with foil.

6



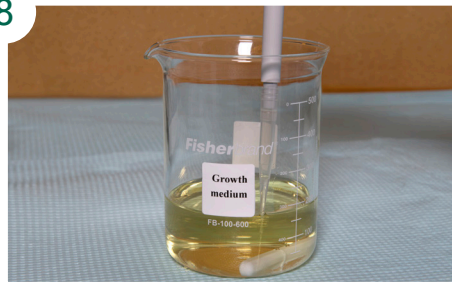
Medium preparation – Step 2: Heat the solution to boil and keep boiling for 3 min. Cool down to room temperature.

7



Medium preparation – Step 3: Add one vial each of chloramphenicol, ascorbic acid, and manganese sulfate stock solution, and 60  $\mu$ L of Tween-80. Mix well for a few minutes.

8



Medium preparation – Step 4: Thaw one vial of frozen microorganism and add 700  $\mu$ L (or amount specified in assay kit) of the inoculum to the medium. Stir gently and cover the beaker with foil.



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