

**SOP: M011****Preparation of Diffco Nutrient agar protocol****Materials and Reagents:**

1. Milli-Q water
2. Beaker, 1 liter
3. Magnetic stir bar
4. Magnetic stir plate
5. Diffco Nutrient Agar (VWR 90000-476)
8. Graduated cylinder, 1 liter
9. Autoclave
10. Bunsen burner
11. Sharpie marker
12. Ziploc bag, one gallon

**Protocol:**

1. \_\_\_\_ Pour 700 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 23.0 g of dehydrated Diffco Nutrient Agar.
4. \_\_\_\_ Make sure powder is completely in solution.
5. \_\_\_\_ Pour medium into 1 liter graduated cylinder.
6. \_\_\_\_ Bring volume to 1000 ml with Milli-Q water.
7. \_\_\_\_ Transfer/aliquot to desired container(s) (note 1).
8. \_\_\_\_ Autoclave on liquid cycle (slow exhaust) at 121°C for 15 minutes.
9. \_\_\_\_ Place sterile medium in 55°C water bath for 30 minutes (note 2).
10. \_\_\_\_ Pour agar into plates (note 3).
11. \_\_\_\_ Remove any bubbles on plates by flaming briefly with a lit bunsen burner.
12. \_\_\_\_ Allow plates to cool and solidify.
13. \_\_\_\_ Label plates and store at 4°C in a Ziploc bag.

**Notes:**

1. It is best to make up the desired amount of agar in each container/volume desired instead of making aliquots from a 1000 ml stock; otherwise, the solution needs to be brought to a boil to completely re-suspend the agar prior to making aliquots. If the agar is not boiled, then it will be unevenly dispersed between containers and the plates will not solidify correctly.
2. Allows the agar solution to cool to a temperature that allows for handling without solidifying the agar.
3. One batch of Nutrient Agar will make approximate nine 15 x 150 mm plates or twenty 15 x 100 mm plates. Plates should be poured thickly to ensure they do not completely dry out when used for culturing of *M. tuberculosis*.