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)
6. Graduated cylinder, 1 liter
7. Autoclave
8. Bunsen burner
9. Sharpie marker
10. 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 dehyrdrated 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.