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| PREPARATION |

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| Preparing MBTA (Middlebrook Top Agar) |

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| **Objective**  To make MBTA for use in plating bacterial lawns. |
| **Background**  Middlebrook Top Agar (MBTA) was developed specifically for use in growing mycobacteria. We use it as part of our final top agar when growing bacterial lawns. |
| **Approximate Time Needed**  ~**15 minutes** active  ~**40 minutes** to autoclave |
| **Materials Needed**  4.7 g 7H9  7.0 g BactoAgar  ddH2O up to 1000 ml  4 drops of anti-bubble  Heat-proof beaker/flask, stir bar, and stir/hotplate for mixing  18 glass bottles for aliquoting |
| **Helpful Tips**   * Preparing MBTA is just the first step in making the final top agar for plating. Check out the **Working Media** protocol for finishing details. * Boiling ensures that all of the agar is evenly distributed. |
| **Procedure**   1. Combine all ingredients in the beaker/flask. 2. Add the stir bar, and put on the stir/hotplate. 3. Stir moderately and heat medium-high until the mixture boils. 4. While still warm/hot, aliquot 50 ml into each of 18 glass bottles. 5. Autoclave on a liquid cycle for an appropriate amount of time. |