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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.
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| **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.
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