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| PREPARATION |

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| Preparing 7H9 |

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| **Objective**  To make stock 7H9 for use in growing mycobacterial cultures and plating mycobacterial lawns. |
| **Background**  7H9 is used as the base for the medium commonly used to grow cultures of mycobacteria, and is added to MBTA to make top agar for plating lawns of bacteria. |
| **Approximate Time Needed**  ~**15 minutes** active  ~**40 minutes** to autoclave |
| **Materials Needed**  4.7 g 7H9 broth base  5 ml 40% glycerol  900 ml ddH2O  Beaker/flask, stir bar, and stir plate for mixing  10 glass bottles for aliquoting |
| **Helpful Tips**   * This broth is NOT ready for use as is. Depending on what it will be used for, 7H9 requires the addition of different supplementary reagents. See the **Working Media** protocol for details. |
| **Procedure**   1. Combine all ingredients in the beaker/flask. 2. Add the stir bar, place on the stir plate, and mix moderately until well combined (~5-10 minutes). 3. Aliquot 90 ml to each of the 10 glass bottles. 4. Autoclave on a liquid cycle for an appropriate amount of time. |