

Andersen Lab Recipes

M9 Buffer

1. Mix the following reagents for a Batch of **4 L of 4X M9 Stock**:

Reagent	Amount Needed: 1X Stock to make 2 L	Amount Needed: 4X Stock to make 4 L
KH ₂ PO ₄	6 g	48 g
Na ₂ HPO ₄	12 g	96 g
NaCl	10 g	80 g
dH ₂ O	up to 2 L	up to 4 L

- First measure all reagents into 3.0 L of distilled water in a 4 L beaker and fully dissolve. Then bring the volume to total of 4 L in a graduated cylinder, and mix thoroughly.
- Transfer the 4X stock to 500 mL bottles using filter sterilization, measuring exactly. Filter Sterilize using the vacuum filtration systems (Thermo 0.45 uM, Rapid Flow PES).

2. Repeat step 1 to make a total of 12 L of 4X Stock. Make sure you have 24 empty 500 ml bottles available (use both the glass and square plastic bottles).

Note: About 32 L of 1X M9 is required per week during maximum Sorter use. Make a **minimum Total of 12 L of 4X Stock** at a time and use one filter. This batch of 4X Stock should last at least 1.5 weeks. Also be sure to have **1 L of 1X M9 in 250 ml bottles on the shelf at all times.**

3. To complement the 4X M9 stocks, prepare 1500 ml of sterile distilled water, pre-measured into 2 L glass bottles.

4. Autoclave filtered M9 and 1500 mL water on the liquid cycle, 30 minutes sterilization.

Note: Make sure to loosen the cap only a little on glass bottles, to maintain final volume. For plastic bottles, do not screw on the cap, but only set it on the bottle, since a tight lid will cause the plastic to deform.

5. When the M9 is cool, add:

MgSO₄, (1 M, filter sterilized): **2 ml** for 500 ml of 4X Stock

(equivalent to 1 ml per 1L of 1X Stock)

To get a 1X M9 working stock of 2 L, mix one of the bottles of 500 ml of 4X Stock with the bottle of 1500 ml sterile water.