

## *Protocol: M9 Buffer*

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This protocol for M9 Buffer is adapted from WormBook.

**Note:** When autoclaving liquid, the rule of thumb is to make sure the final volume of your liquid is no more than ½ the max volume of the container (e.g., no more than 500 mL of liquid in a 1 L Pyrex bottle.)

1. To an autoclavable bottle, add:

Reagent	Amount to add per 500ml final volume	Amount to add per 250ml final volume
KH <sub>2</sub> PO <sub>4</sub>	1.5 g	0.75 g
Na <sub>2</sub> HPO <sub>4</sub>	3 g	1.5 g
NaCl	2.5 g	1.25 g

- i. Weigh out and add dry reagents to autoclavable Pyrex bottle.
  - ii. Using a graduated cylinder, measure out the appropriate volume of diH<sub>2</sub>O.
  - iii. Add diH<sub>2</sub>O to the dry reagents and swirl.
2. Autoclave the mixture (**You MUST get trained on how to use the autoclave before doing this!**)
    - i. Make sure the screw-caps are **LOOSLEY** screwed on, allowing gas to escape the bottle.
    - ii. Place a piece of autoclave tape across the cap, making sure one end is taped to the glass bottle.
    - iii. Autoclave for 25 minutes (use “SLOW EXHAUST” setting for liquids).
  3. After autoclaving, add the following volumes of 1M MgSO<sub>4</sub>:

Reagent	Amount to add per 500ml final volume	Amount to add per 250ml final volume
1M MgSO <sub>4</sub>	500µl	250µl