

## **5x M9 Salts**

### **Overview**

This protocol covers the creation of 5x M9 salts for M9 minimal media. M9 salts and remaining media components are made separately, as the salts cannot be autoclaved with the other components due to the potential of unwanted reactions<sup>i</sup>. The procedure section of this protocol assumes you are making 500mL of salts, but the volumes can be scaled up or down as appropriate.

### **Materials**

- 1L Autoclavable glass bottle
  - o 200mL of 5x salts are used for 1L of M9
- Millipore Water
- ddH<sub>2</sub>O
- Na<sub>2</sub>HPO<sub>4</sub> (Sodium phosphate dibasic)
  - o Note, compound is hygroscopic, close lid tightly.
  - o 30g/L<sup>ii</sup>
- NaCl (Sodium chloride)
  - o 2.5g/L
- KH<sub>2</sub>PO<sub>4</sub> (Potassium phosphate monobasic)
  - o 15g/L
- NH<sub>4</sub>Cl (Ammonium chloride)
  - o 5g/L

### **Procedure**

1. In a 1L glass bottle, add: 1.25g NaCl, 2.5g NH<sub>4</sub>Cl, 7.5g KH<sub>2</sub>PO<sub>4</sub>, 15g Na<sub>2</sub>HPO<sub>4</sub>.
2. Add Millipore water to 450mL, dissolve
3. Adjust volume to 500mL with Millipore water.
4. Autoclave to sterilize.

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<sup>i</sup> This is especially true with any organic components of the media, as the presence of high heat and salts will lead to degradation (e.g. glucose will be caramelized or undergo the Maillard reaction if autoclaved with amino acids.)

<sup>ii</sup> For why concentrations are given in g/L and not M, see ([https://openwetware.org/wiki/Talk:M9\\_salts](https://openwetware.org/wiki/Talk:M9_salts))