

Minimal Medium – M9

Prepare and autoclave **10x** salts w/o NH₄Cl

<u>Component</u>	<u>FW</u>	<u>g (/L)</u>
Na ₂ HPO ₄	132	60
KH ₂ PO ₄	74.5	30
NaCl	68.1	5

Adjust pH to 7.0 (should be close already)

To a 2.5L baffled flask add:

100mL of 10x salts (from above)

900mL ddH₂O

cover with foil

label appropriately

autoclave (30 Liq cycle)(1 hr)

store in warm room as the 60mL O/N culture grows

on the day of the large-scale growth add the following to each flask (1L solution):

<u>Component</u>	<u>FW</u>	<u>(/L)</u>
CaCl ₂	147.02	50μL of 1M filtered stock solution
MgSO ₄	246.48	1mL of 1M filtered stock solution
Gibco Vitamin Solution		10mL

¹⁵NH₄Cl 1.0 g solid (fill a 1.5mL Eppendorf tube)
glucose 2.0 g for ¹³C or 2.5-3.0 g for ¹²C growth

add appropriate antibiotics:

Kan	1mL of 20mg/mL filtered stock (H ₂ O)
Cap	1mL of 34mg/mL filtered stock (EtOH)
Amp	1mL of 100mg/mL filtered stock (H ₂ O)

Inoculate with 10mL of O/N LB growth

Grow to OD₆₀₀ = 0.5 – 0.8 and induce with 1mL of 1M IPTG stock

Optional Procedures/Ingredients

Addition of 20mL of 100mM FeCl₃ per liter of M9

2-3 step rich-to-minimal acclimation of culture