

**M4 Medium Overall recipe:**

Add the following to the correct amount of distilled water

Component	Conc (mM)	MW (g/mol)	add per 1L (g)	add per 10L (g)
K <sub>2</sub> HPO <sub>4</sub>	1.27	174.18	0.221	2.21
KH <sub>2</sub> PO <sub>4</sub>	0.73	136.09	0.099	0.99
NaHCO <sub>3</sub>	2.00	84.01	0.168	1.68
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	9.00	132.14	1.189	11.89
NaCl	125.00	58.44	7.305	73.05
Hepes	5.00	238.3	1.192	11.92
Yeast extract			0.5	5.0
Tryptone			0.5	5.0
100X minerals			10 mL	100 mL
100X CaCl <sub>2</sub>			10 mL	100 mL
40% sodium lactate	<b>ADD AFTER</b>	<b>AUTOCLAVING!</b>	4.3 mL	43 mL

**For 100X Mineral Mix:**

Add the following to 1 L distilled water. Store at 4C.

component	final mM	g/mol	final mg/L	100X g/L
Na <sub>2</sub> EDTA	0.0672	336.24	22.595328	2.260
MgSO <sub>4</sub> *7H <sub>2</sub> O	1.01	246.47	248.9347	24.89
MnSO <sub>4</sub> *4H <sub>2</sub> O	0.0013	223.07	0.289991	0.029
NaCl	0.01	58.44	0.5844	0.058
FeCl <sub>2</sub>	0.0054	126.751	0.6844554	0.068
CoCl <sub>2</sub>	0.005	129.839	0.649195	0.065
ZnSO <sub>4</sub> *7H <sub>2</sub> O	0.001	287.53	0.28753	0.029
CuSO <sub>4</sub> *5H <sub>2</sub> O	0.0002	249.7	0.04994	0.005
H <sub>3</sub> BO <sub>3</sub>	0.0566	61.83	3.499578	0.350
Na <sub>2</sub> MoO <sub>4</sub>	0.0039	205.92	0.803088	0.080
NiCl <sub>2</sub> *6H <sub>2</sub> O	0.005	237.69	1.18845	0.119
Na <sub>2</sub> SeO <sub>4</sub>	0.0015	188.94	0.28341	0.028

**For 100X CaCl<sub>2</sub> mixture:**

Add 7.13 g CaCl<sub>2</sub>\*2H<sub>2</sub>O to 1L H<sub>2</sub>O. Store at 4C.

**For 40% sodium lactate:**

Dilute 60% lactate solution (as ordered) to 40% by pouring 333 mL 60% solution into a graduated cylinder and bringing the volume to 500 mL with distilled water. Mix well, filter sterilize and store at 4C in the filter bottle.