

Main sol. 930

KH_2PO_4	0.20	g
$\text{MgCl}_2 \times 6 \text{ H}_2\text{O}$	0.10	g
NH_4Cl	1.00	g
KCl	0.20	g
NaCl	80.00	g
Na_2CO_3	6.83	g
NaHCO_3	3.83	g
$\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$	0.20	g
Yeast extract	0.20	g
Histidine	5.00	g
Wolin's vitamin solution	2.00	ml
Modified Wolin's mineral solution II	1.00	ml
Resazurin	1.00	mg
Distilled water	1000.00	ml

1. Final pH 8.9

2. Prepare the medium without adding the vitamins, histidine, yeast extract, NH_4Cl , Na_2CO_3 , and NaHCO_3 using anaerobic conditions, under nitrogen. If the medium has been boiled to remove oxygen add the NH_4Cl , Na_2CO_3 , and NaHCO_3 after the medium had cooled. Dispense into tubes stopper with rubber stoppers (serum tubes or bottles, or screw capped tubes). Autoclave, and to the cooled medium add the vitamins (filter sterilised under N_2), histidine, yeast extract, and $\text{Na}_2\text{S} \times 9 \text{ H}_2\text{O}$ from anaerobic, sterile stock solutions.