

Main sol. 718

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	4.00	g
MgSO ₄ x 7 H ₂ O	3.45	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	18.00	g
Modified Wolin's mineral solution	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 7 H ₂ O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
D-Glucose	5.00	g
Yeast extract	0.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose, yeast extract, vitamins and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add bicarbonate, adjust pH to 6.5 and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add glucose, yeast extract, vitamins, and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins should be sterilized by filtration. Prior to inoculation adjust pH of complete medium to 6.5 - 6.7, if necessary.