

Main sol. 689

KCl	0.33	g
MgCl ₂ x 6 H ₂ O	6.00	g
MgSO ₄ x 7 H ₂ O	1.00	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
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (0.1% w/v)	2.00	ml
Yeast extract (Difco)	1.00	g
Resazurin	1.00	mg
L-Cysteine HCl x H ₂ O	0.50	g
NaHCO ₃	2.00	g
Glucose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, bicarbonate, glucose and vitamins, then sparge medium with 100% N₂ gas to make it anoxic. Add cysteine and sodium bicarbonate, then adjust pH to 7.5. Dispense medium in anoxic Hungate-type tubes under 100% N₂ gas and autoclave. A precipitate forms which will redissolve slowly over about 3 days. Before use inject separately sterile glucose solution (20% w/v) and vitamins prepared under 100% N₂ gas atmosphere. Vitamins should be sterilized by filtration. Check medium pH and adjust pH to 7.6, if necessary.