

Main sol. 1539

K ₂ HPO ₄	0.05	g
KH ₂ PO ₄	0.09	g
NH ₄ Cl	0.25	g
MgSO ₄ x 7 H ₂ O	0.25	g
CaCl ₂ x 2 H ₂ O	0.15	g
Yeast extract	1.00	g
Trace element solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	2.00	g
D-Glucose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, glucose, vitamins, cysteine and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas (vitamins are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use adjust pH of complete medium to 7.0 - 7.5, if necessary.