

Main sol. 298b

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	1.00	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Trace element solution SL-10	1.00	ml
Na-acetate	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Na ₂ -glyoxalate	0.60	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

1. Dissolve ingredients except carbonate, glyoxalate, 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 in anoxic Hungate-type tubes or serum vials under the same gas atmosphere and autoclave. Add glyoxalate (sterilized by filtration), vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas 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 7.2 - 7.5.

2. Note: When growth has started, feed culture once or twice with the same amount of glyoxalate.