

Main sol. 317

NH ₄ Cl	1.00	g
K ₂ HPO ₄	0.40	g
MgCl ₂ x 6 H ₂ O	0.20	g
Minerals solution	50.00	ml
Modified Wolin's mineral solution	10.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Methanol	5.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	950.00	ml

Dissolve ingredients except carbonate, methanol, 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 vilas and autoclave. Add methanol, 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 6.8 - 7.0, if necessary.