

Main sol. 525a

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	2.75	g
MgSO ₄ x 7 H ₂ O	3.45	g
NH ₄ Cl	0.25	g
K ₂ HPO ₄ x 3 H ₂ O	0.14	g
CaCl ₂ x 2 H ₂ O	0.14	g
NaCl	150.00	g

Modified Wolin's mineral solution

Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Na ₂ CO ₃	0.50	g
Trimethylamine-HCl	5.00	g

Wolin's vitamin solution (10x)

L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonates, trimethylamine, 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 same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete medium by adding sterile anoxic stock solutions of trimethylamine, vitamins (sterilized by filtration), cysteine and sulfide prepared under 100% N₂ gas atmosphere and bicarbonate and carbonate from sterile anoxic stock solutions prepared under 80% N₂ and 20% CO₂ gas mixture. Adjust pH of complete medium to 6.9 - 7.0, if necessary.