

Main sol. 924

NaCl	10.00	g
MgCl ₂ x 6 H ₂ O	1.00	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.40	g
K ₂ HPO ₄	0.40	g
NH ₄ Cl	1.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	4.00	g
Na-acetate	1.60	g
Na-formate	6.80	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, acetate, formate, vitamins and reducing agents), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Add solid bicarbonate, adjust pH to 6.8 - 7.0, dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic serum bottles and autoclave. Add acetate, formate, vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The pH of the complete medium should be 7.2.