

Main sol. 1526d

NaCl	60.00	g
MgCl ₂ x 6 H ₂ O	6.00	g
KCl	1.50	g
Na ₂ SO ₄	1.00	g
NH ₄ Cl	1.00	g
CaCl ₂ x 2 H ₂ O	0.40	g
K ₂ HPO ₄	0.40	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Na-acetate	1.60	g
Na ₂ -fumarate	6.40	g
Seven vitamins solution	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, acetate, fumarate, 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 acetate, fumarate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas (fumarate and 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 6.8 - 7.0, if necessary.