

Main sol. 1526c

NaCl	60.00	g
MgSO ₄ x 7 H ₂ O	6.00	g
KCl	1.50	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	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
Yeast extract (BD Bacto)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Na-pyruvate	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, pyruvate, vitamins, 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 pyruvate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas (pyruvate 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 7.3 - 7.5, if necessary.

2. Note: Use at least 5-10% (v/v) inoculum.