

Main sol. 1030

NH ₄ Cl	1.00	g
MgCl ₂ x 6 H ₂ O	1.00	g
CaCl ₂ x 2 H ₂ O	0.40	g
KH ₂ PO ₄	0.40	g
Wolfe's mineral elixir	1.00	ml
Yeast extract	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Na-crotonate solution (1 M)	20.00	ml
2-Mercaptoethanesulfonic acid (coenzyme M)	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, crotonate, coenzyme M, 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 same gas atmosphere into Hungate-type tubes or serum vials (e.g., 5 ml medium in Balch-type tubes) and autoclave. Add crotonate, coenzyme M, vitamins 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. Stock solutions of crotonate, coenzyme M and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2.

2. Note: Use 10% (v/v) as inoculum.