

Main sol. 516

NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
KCl	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g

Trace element solution SL-10	1.00	ml
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Yeast extract	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Cellobiose	5.00	g

Wolin's vitamin solution (10x)	1.00	ml
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Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except vitamins, carbonate, cellobiose 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. Prior to inoculation add vitamins, cellobiose 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 vitamins and cellobiose should be sterilized by filtration. The pH of the complete medium should be at 7.1 - 7.3.