

Main sol. 1318

MgCl ₂ x 6 H ₂ O	0.40	g
CaCl ₂ x 2 H ₂ O	0.10	g
NH ₄ Cl	0.10	g
KH ₂ PO ₄	0.20	g
KCl	0.50	g
Yeast extract	0.20	g
Na-acetate	0.09	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Seven vitamins solution	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
DL-Dithiothreitol	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, cysteine, and DTT) and 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 to 30% of their volume and autoclave. Add vitamins (sterilized by filtration), cysteine, and DTT 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. The pH of the complete medium should be 7.0.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 100% H₂ gas.