

Main sol. 507

KCl	0.33	g
MgCl ₂ x 6 H ₂ O	0.52	g
CaCl ₂ x 2 H ₂ O	0.29	g
NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
Yeast extract	0.05	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	20.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, yeast extract, vitamins and sulfide, then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add yeast extract, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Neutralize the sodium sulfide with sterile H₂SO₄ (see medium 29). After completing the medium adjust pH to 6.8-7.0.
2. Inoculated vessels are pressurized with sterile carbon monoxide gas to 2 bar overpressure.