

Main sol. 901a

NH ₄ Cl	0.33	g
KCl	0.33	g
KH ₂ PO ₄	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
Na ₂ SO ₄	2.80	g
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	1000.00	ml

Dissolve ingredients except yeast extract, vitamins and sulfide. Adjust pH to 4.5 with H₂SO₄ and sparge medium with 100% CO₂ gas 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 yeast extract, vitamins (sterilized by filtration), and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The sulfide stock solution (3% w/v) should be neutralized before use (see medium 28). The pH of the complete medium should be at 4.5.