

Main sol. 778b

KH ₂ PO ₄	0.33	g
NH ₄ Cl	0.33	g
KCl	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
NiCl ₂ x 6 H ₂ O (0.1% w/v)	0.20	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	2.50	g
CaCl ₂ x 2 H ₂ O	0.33	g
Na ₂ -fumarate	1.60	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, calcium chloride, fumarate, thiosulfate, vitamins and sulfide). Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, add solid bicarbonate and adjust pH to 7.2. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add calcium chloride, fumarate, thiosulfate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere. Stock solutions of fumarate, thiosulfate and vitamins are sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.