

Main sol. 508

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-4

Sodium resazurin (0.1% w/v)	10.00	ml
Na ₂ CO ₃	0.50	ml
Na-pyruvate	1.00	g
Yeast extract	2.50	g
	0.05	g

Seven vitamins solution

	1.00	ml
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Wolin's vitamin solution (10x)

	1.00	ml
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, pyruvate, yeast extract, vitamins 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. Add pyruvate, yeast extract, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Stock solutions of pyruvate and vitamins are sterilized by filtration. After completing the medium adjust pH to 6.8-7.0 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v), if necessary.