

Main sol. 480

NH ₄ Cl	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
KCl	0.33	g
KH ₂ PO ₄	0.33	g

Trace element solution SL-10	1.00	ml
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Yeast extract	0.10	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powder)	10.00	g
Na-acetate	5.00	g
NaHCO ₃	2.50	g

Wolin's vitamin solution	10.00	ml
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Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfur, acetate, bicarbonate, vitamins and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Adjust pH to 5.9, dispense under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and sterilize by heating for 2-3 hours in a boiling water bath on each of 3 successive days. Add acetate, vitamins (sterilized by filtration) and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to use adjust pH of complete medium to 6.8 - 7.0, if necessary.