

Main sol. 135b

NH ₄ Cl	1.00	g
KH ₂ PO ₄	0.33	g
K ₂ HPO ₄	0.45	g
MgSO ₄ x 7 H ₂ O	0.10	g
Modified Wolin's mineral solution	20.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Na-syringate solution (0.3 M)	15.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	970.00	ml

1. Dissolve ingredients except for carbonate, Na-syringate solution, vitamins, and cysteine, bring to the boil and cool to room temperature under 80% N₂ and 20% CO₂ gas atmosphere. Thereafter, dispense under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Add Na-syringate solution, vitamins, and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins should be sterilized by filtration. The pH of the completed medium should be at 7.4.
2. Note: After growth has been started add an additional amount of Na-syringate to reach a final concentration of 2.00 g/l.