

Main sol. 135

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
NaHCO ₃	10.00	g
D-Fructose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine and sulfide, bring to the boil and cool to room temperature under 80% N₂ and 20% CO₂ gas mixture. Add bicarbonate (solid) and equilibrate the medium with the gas until a pH of around 7.4 is reached. Then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Before use adjust the pH to 8.0 - 8.2 by adding a sterile anoxic stock solution of sodium carbonate (5% w/v) prepared under 80% N₂ and 20% CO₂ gas mixture (c. 0.25 ml per 10 ml medium) and add fructose, vitamins (sterilized by filtration), cysteine and sulfide from anoxic sterile stock solutions prepared under 100% N₂.

2. Note: For autotrophic growth D-fructose is omitted and a gas atmosphere of 80% H₂ and 20% CO₂ is used.