Main sol. 135c

NH_4Cl	1.00	g
KH_2PO_4	0.33	g
K_2HPO_4	0.45	g
$MgSO_4$ x 7 H_2O	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
$NaHCO_3$	1.00	g
D-Fructose (optional)	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add fructose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 6.5.

2. Note: For autotrophic growth fructose is omitted and a gas atmosphere of 80% H_2 and 20% CO_2 is used.