

Main sol. 710

Tryptone (BD Bacto)	1.00	g
Peptone (meat)	1.00	g
Yeast extract (BD Bacto)	1.00	g
K ₂ HPO ₄	1.60	g
NaH ₂ PO ₄ x 2 H ₂ O	1.00	g
NH ₄ Cl	0.50	g
MgSO ₄ x 6 H ₂ O	0.16	g
Trace element solution SL-11	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
CaCl ₂ x 2 H ₂ O	0.06	g
NaHCO ₃	1.00	g
D-Glucose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.0 and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add calcium chloride, glucose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Add bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are prepared under 100% N₂ gas and sterilized by filtration. The pH of the complete medium should be at 7.0.

2. Note: Supplementation of medium with 10.00 g/l MOPS buffer (pH 6.9 - 7.0; 10% (w/v) anoxic stock solution) may enhance the buffer capacity of the medium.