

Main sol. 880

KH ₂ PO ₄	0.30	g
NaCl	0.60	g
MgCl ₂ x 6 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.08	g
NH ₄ Cl	1.00	g
Trace element solution SL-11	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
KHCO ₃	3.50	g
L-Cysteine HCl x H ₂ O	0.60	g
Methanol (50% v/v)	6.00	ml
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, cysteine, methanol, vitamins and sulfide), sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then add solid bicarbonate and cysteine and equilibrate the medium with the gas mixture to reach a pH of 7.0. Dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. To the autoclaved medium add methanol (50% v/v solution), vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins should be sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.