Main sol. 120d

K_2HPO_4	0.35	g
KH_2PO_4	0.23	g
NH_4Cl	0.50	g
$MgCl_2$ x 6 H_2O	10.00	g
$CaCl_2$ x 2 H_2O	0.15	g
NaCl	23.00	g
$FeSO_4$ x 7 H_2O solution (0.1% w/v)	2.00	ml
Trace element solution SL-10	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.00	g
Methanol (50% v/v)	20.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except carbonate, vitamins, methanol, cysteine and sulfide) and sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Methanol (50% v/v stock solution) and the reducing agents are each autoclaved separately under 100% N_2 gas atmosphere as concentrated solutions in tightly closed tubes. Carbonate is prepared under 80% N_2 and 20% CO_2 gas mixture and autoclaved separately. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Appropriate volumes of the stock solutions are injected into the sterile medium with hypodermic syringes. Adjust pH of the complete medium to 6.8 - 7.0, if necessary.