Main sol. 711

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	0.60	g
Yeast extract	0.50	g
Na-acetate x 3 H_2O	0.50	g
Trace element solution SL-10	1.50	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
3,4,5-Trimethoxycinnamic acid (TMC)	1.19	g
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, TMC and reducing agents) and sparge medium with 80% N_2 and 20% CO_2 gas mixture 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 autoclaving add vitamins, TMC, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas atmosphere. The vitamin solution should be sterilized by filtration. The stock solution of TMC (6% w/v) should be neutralized with NaOH prior to sterilization by filtration. Adjust pH of complete medium to 7.0, if necessary.

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