Main sol. 1616

NaCl	20.00	g
NH_4Cl	0.54	g
KH_2PO_4	0.10	g
$MgCl_2$ x 7 H_2O	4.00	g
$CaCl_2$ x 2 H_2O	1.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na_2CO_3	1.50	g
Na-pyruvate	2.75	g
Wolin's vitamin solution (10x)	2.00	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, pyruvate, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Distribute medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add pyruvate, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pyruvate and vitamin solutions should be sterilized by filtration. Check pH of complete medium and adjust to 7.2, if necessary.