899. THERMOANAEROBACTER SUBTERRANEUS MEDIUM

NH_4Cl	1.00	g
K_2HPO_4	0.30	g
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$MgCl_2$ x 6 H_2O	1.00	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.20	g
NaCl	0.60	g
Trace element solution (see medium 141)	10.00	ml
Yeast extract	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
D-Glucose	4.00	g
$Na_2S_2O_3$ x 5 H_2O	2.50	g
L-Cysteine-HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.45	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, glucose, thiosulfate, cysteine and sulfide), then 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. Add glucose, thiosulfate (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The pH of the complete medium should be at 7.0.