664a. PSEUDOTHERMOTOGA LETTINGAE MEDIUM

NH_4Cl	1.0	g
K_2HPO_4	0.3	g
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$MgCl_2$ x 6 H_2O	0.2	g
$CaCl_2$ x 2 H_2O	0.1	g
KCl	0.1	g
NaCl	10.0	g
Trace element solution (see medium 141)	10.0	ml
Yeast extract	0.5	g
Na-resazurin solution (0.1% w/v)	0.5	ml
Na_2CO_3	2.0	g
$Na_2S_2O_3$ x 5 H_2O	5.0	g
D-Glucose	4.0	g
L-Cysteine-HCl x H_2O	0.5	g
Na_2S x 9 H_2O	0.5	g
Distilled water	1000.0	ml

Dissolve ingredients except carbonate, thiosulfate, glucose and sulfide. Sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add thiosulfate, glucose, 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 pH of the complete medium should be adjusted to 7.0, if necessary.