1526e. LIMIHALOGLOBUS MEDIUM

NaCl	60.0	g
$MgCl_2$ x 6 H_2O	6.0	g
KCl	1.5	g
Na_2SO_4	1.0	g
NH_4Cl	1.0	g
$CaCl_2$ x 2 H_2O	0.4	g
K_2HPO_4	0.4	g
Trace elements solution (see medium 141)	10.0	ml
Na-resazurin solution (0.1% w/v)	0.5	ml
Sulfur, powder	10.0	g
Na_2CO_3	1.5	g
D-Glucose	1.0	g
Yeast extract	1.0	g
Vitamins solution (see medium 141)	10.0	ml
L-Cysteine-HCl x H_2O	0.3	g
Na_2S x 9 H2O	0.3	g
Distilled water	1000.0	ml

Dissolve ingredients except sulfur, carbonate, glucose, yeast extract, vitamins, cysteine and DTT, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 – 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur and autoclave at **110°C** for 20 min. Add glucose, yeast extract, vitamins, cysteine and DTT from sterile anoxic stock solutions prepared under 100% N_2 gas (vitamins and DTT are sterilized by filtration) and carbonate from a sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Prior to use adjust pH of complete medium to 7.3 - 7.5, if necessary.

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