945: MARINITOGA PIEZOPHILA MEDIUM

Final pH: 6.0
Final volume: 1000 ml

NH_4Cl	1.00	g
$MgCl_2$ x 6 H_2O	0.20	g
$CaCl_2$ x 2 H_2O	0.10	g
KCl	0.10	g
NaCl	30.00	g
Na-acetate	0.83	g
MES (SIGMA)	1.95	g
Yeast extract	5.00	g
Trypticase peptone (BD BBL)	5.00	g
K_2HPO_4	0.30	g
KH_2PO_4	0.30	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	g
L-Cysteine HCl x H_2O	0.30	g
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients except sulfur, cysteine and sulfide. Sparge medium with 100% N_2 gas for at least 30 - 45 min to make it anoxic and adjust pH to 6.0 with NaOH. Distribute the medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur. For sterilization heat the cultivation vessels containing medium plus sulfur in boiling water for 2 - 3 hours on each of 3 successive days! Prior to inoculation add cysteine and sulfide and from sterile anoxic stock solutions prepared under 100% N_2 gas.

2. After inoculation pressurize tubes to 2 bar overpressure with sterile 100% N_2 gas.

For DSM 14283: 5.00 g/l maltose can be used as substrate instead of sulfur. Maltose is added to autoclaved medium from an anoxic stock solution, sterilized by filtration.

For DSM 17373: Adjust pH of complete medium to 5.5. After inoculation pressurize tubes to 2 bar overpressure with sterile 80% H_2 and 20% CO_2 gas mixture.