

945. MARINITOGA PIEZOPHILA MEDIUM

NH ₄ Cl	1.00	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	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 ₂ HPO ₄	0.30	g
KH ₂ PO ₄	0.30	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
L-Cysteine-HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients except sulfur, cysteine and sulfide. Sparge medium with 100% N₂ gas for at least 30 – 45 min to make it anoxic and adjust pH to 6.0 with NaOH. Distribute the medium under 100% N₂ 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% N₂ gas. After inoculation pressurize tubes to 2 bar overpressure with sterile 100% N₂ 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% H₂ and 20% CO₂ gas mixture.