

1254. NAUTILIA NITRATIREDUCTENS MEDIUM

NaCl	20.00	g
MgSO ₄ x 7 H ₂ O	3.50	g
MgCl ₂ x 6 H ₂ O	2.75	g
CaCl ₂ x 2 H ₂ O	0.75	g
KH ₂ PO ₄	0.50	g
KCl	0.33	g
NaBr	0.05	g
H ₃ BO ₃	0.02	g
(NH ₄) ₂ SO ₄	0.01	g
SrCl ₂ x 6 H ₂ O solution (0.1% w/v)	7.00	ml
NiCl ₂ x 6 H ₂ O solution (0.1% w/v)	2.00	ml
KI solution (0.01% w/v)	0.50	ml
Na ₂ WO ₄ x 2 H ₂ O solution (0.1% w/v)	0.10	ml
Modified Wolin's mineral solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ S x 9 H ₂ O	0.50	g
KNO ₃	1.00	g
PIPES (SIGMA)	3.80	g
Distilled water	1000.00	ml

Dissolve ingredients (except sulfide, nitrate and PIPES), sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add sulfide from a freshly prepared anoxic stock solution (5% w/v), wait until the medium is colorless and adjust pH to 7.0 while gassing the headspace only. Dispense medium under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add nitrate from a sterile anoxic stock solution prepared under 100% N₂ gas and PIPES from a sterile 0.5 M stock solution adjusted to pH 7.0 and prepared under 100% N₂ gas. The pH of the complete medium should be 7.0. After inoculation add sterile 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.