

819: SEDIMINISPIROCHAETA SMARAGDINAЕ MEDIUM

NH ₄ Cl	1.00	g
K ₂ HPO ₄	0.30	g
KH ₂ PO ₄	0.30	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
KCl	0.20	g
NaCl	50.00	g
Yeast extract	5.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ CO ₃	1.00	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, carbonate and sulfide), sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Then, add cysteine, distribute medium under 80% N₂ and 20% CO₂ gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Check pH of complete medium and adjust to pH 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.