

795a: LEBETIMONAS MEDIUM

Synthetic seawater (2 x conc.)	500.00	ml
NH ₄ Cl	1.25	g
KH ₂ PO ₄	0.50	g
NiCl ₂ x 6 H ₂ O (0.1% w/v)	3.00	ml
Modified Wolin's mineral solution	10.00	ml
Na-formate	0.20	g
Tryptone (BD Bacto)	1.00	g
Yeast extract (OXOID)	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Sulfur, powdered	10.00	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	500.00	ml

1. Dissolve ingredients (except bicarbonate, vitamins, sulfur, and sulfide), then sparge medium with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic and adjust pH to 5.5 with H₂SO₄. Dispense under the same gas atmosphere in suitable culture vessels (e.g. 20 ml of the medium in 100 ml serum bottles) and autoclave. Steam sulfur for 3 hours on each of 3 successive days (see medium 35). Aseptically mix the sterilized sulfur with the sterile medium while retaining anoxic conditions. Add vitamins from an anoxic stock solution prepared under 100% N₂ gas atmosphere and sterilized by filtration and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Prior to inoculation add sulfide from a sterile anoxic stock solution prepared under 100% N₂ gas atmosphere. Check pH and adjust to 5.5, if necessary.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

Synthetic seawater (2 x conc.) (from medium 795)

NaCl	55.40	g
SrCl ₂ x 6 H ₂ O	0.03	g
MgSO ₄ x 7 H ₂ O	14.00	g
KI	0.10	mg
MgCl ₂ x 6 H ₂ O	11.00	g
Na ₃ -citrate	20.00	mg
KCl	1.30	g
CaCl ₂ x 2 H ₂ O	1.50	g
NaBr	0.20	g
H ₃ BO ₃	0.06	g
Distilled water	1000.00	ml

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

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml