795a. LEBETIMONAS MEDIUM

Synthetic seawater (2 x conc.) (see medium 795) 500.00 ml
NH₄Cl 1.25 g
KH₂PO₄ 0.50 g
NiCl₂ x 6 H₂O solution (0.1% w/v) 3.00 ml
Trace element solution (see medium 141) 10.00 ml
Na-formate 0.20 g
Tryptone (BD Bacto) 1.00 g
Yeast extract (OXOID) 1.00 g
Na-resazurin solution (0.1% w/v) 0.50 ml
NaHCO₃ 2.00 g
Vitamin solution (see medium 141) 10.00 ml
Sulfur, powdered 10.00 g
Na₂S x 9 H₂O 0.50 g
Distilled water 500.00 ml

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 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. 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. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.