

817. THERMOSPHERA MEDIUM

MgCl ₂ x 6 H ₂ O	2.20	g
NaCl	0.90	g
KCl	17.00	mg
NH ₄ Cl	12.00	mg
CaCl ₂ x 2 H ₂ O solution (0.1% w/v)	7.00	ml
K ₂ HPO ₄ x 3 H ₂ O solution (0.1% w/v)	7.00	ml
FeCl ₃ solution (0.01% w/v in 0.2 N HCl)	0.50	ml
Yeast extract (BD Bacto)	1.00	g
Peptone (BD Bacto)	1.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	0.50	g
Vitamin solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, vitamins and sulfide. Sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins should be sterilized by filtration. The pH of the complete medium should be adjusted to 6.5. After inoculation pressurize tubes or bottles (heavy walled) to 2 bar overpressure with sterile 80% N₂ and 20% CO₂ gas mixture.