

731. THERMOSYNTROPHA MEDIUM

K ₂ HPO ₄	0.30	g
KCl	0.30	g
NaCl	0.50	g
NH ₄ Cl	1.00	g
MgCl ₂ x 6 H ₂ O	0.30	g
CaCl ₂ x 2 H ₂ O	0.05	g
Trace element solution (see medium 141)	10.00	ml
Yeast extract	10.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	3.00	g
Na ₂ CO ₃	3.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.15	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except bicarbonate, carbonate, vitamins, cysteine and sulfide. Sparge medium with 100% N₂ gas for 30 – 45 min to make it anoxic, then dissolve carbonate and bicarbonate and adjust pH of medium to 8.5 using HCl. Dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins should be sterilized by filtration.