

731: THERMOSYNTROPH A 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
Modified Wolin's mineral solution	10.00	ml
Yeast extract	10.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	3.00	g
Na ₂ CO ₃	3.00	g
Wolin's vitamin solution (10x)	1.00	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.

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.

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