

613: THERMOTOGA TF(C) MEDIUM

NH ₄ Cl	0.50	g
K ₂ HPO ₄	1.60	g
Na ₂ HPO ₄ x H ₂ O	1.00	g
NaCl	7.00	g
MgCl ₂ x 6 H ₂ O	1.40	g
MgSO ₄ x 7 H ₂ O	1.96	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
D-Glucose	3.00	g
CaCl ₂ x 2 H ₂ O	0.06	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except glucose, calcium chloride, vitamins and reducing agents), adjust pH to 6.5 - 6.8 and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Add glucose, calcium chloride, vitamins (sterilized by filtration), sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere.

For DSM 4359: Replace glucose with 0.50 g/l soluble starch added to the autoclaved medium from a sterile anoxic stock solution prepared under 100% N₂ gas.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic 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