

1057: ETHANOLIGENES MEDIUM

Tryptone (BD BBL)	4.00	g
Beef extract (BD BBL)	2.00	g
Yeast extract (OXOID)	1.00	g
NaCl	4.00	g
K ₂ HPO ₄	1.50	g
FeSO ₄ x 7 H ₂ O	0.10	g
MgCl ₂ x 6 H ₂ O	0.20	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
D-Glucose	10.00	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

Dissolve ingredients (except cysteine, glucose and vitamins), sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Add cysteine and adjust pH to 6.0, then dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Prior to inoculation add glucose and vitamins from sterile anoxic stock solutions prepared under 100% N₂ gas. The vitamin solution should be sterilized by filtration. Adjust pH of complete medium to 6.0, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
FeCl ₂ x 4 H ₂ O	1.00	g
MnCl ₂ x 4 H ₂ O	0.10	g
CoCl ₂ x 6 H ₂ O	0.03	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnCl ₂	0.10	g
CuCl ₂	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
NiCl ₂ x 6 H ₂ O	0.10	g
NaCl	1.00	g
Na ₂ SeO ₃ x 5 H ₂ O	0.03	g
Na ₂ WO ₄ x 2 H ₂ O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

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