

1051: TEPIDANAEROBACTER MEDIUM

KH ₂ PO ₄	0.14	g
MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.15	g
NH ₄ Cl	0.54	g
Trace element solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Yeast extract	2.30	g
Wolin's vitamin solution	2.00	ml
D-Glucose	2.20	g
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 carbonate, yeast extract, vitamins, glucose, cysteine and sulfide), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, vitamins (sterilized by filtration), glucose, cysteine 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 mixture. Adjust pH of the complete medium to 7.0-7.2, if necessary.

For DSM 21860: Omit glucose, increase amount of ammonium chloride to 2.50 g/l, decrease amount of yeast extract to 0.20 g/l and supplement medium with 1.00 ml/l selenite-tungstate solution (see medium 385). As substrate add 1.10 g/l betaine to the autoclaved medium from an anoxic stock solution sterilized by filtration.

Trace element solution (from medium 144)

Nitrilotriacetic acid (NTA)	12.80	g
FeCl ₂ x 4 H ₂ O	0.20	g
MnCl ₂ x 4 H ₂ O	0.10	g
CoCl ₂ x 6 H ₂ O	0.17	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	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
NaCl	1.00	g
Na ₂ SeO ₃ x 5 H ₂ O	0.03	g
Distilled water	1000.00	ml

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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 (from medium 141)

Biotin	2.00	mg
Folic acid	2.00	mg
Pyridoxine hydrochloride	10.00	mg
Thiamine HCl	5.00	mg
Riboflavin	5.00	mg
Nicotinic acid	5.00	mg
Calcium D-(+)-pantothenate	5.00	mg
Vitamin B ₁₂	0.10	mg
p-Aminobenzoic acid	5.00	mg
(DL)-alpha-Lipoic acid	5.00	mg
Distilled water	1000.00	ml