

1104: DETHIOBACTER MEDIUM

NaCl	6.00	g
K ₂ HPO ₄	0.50	g
NaHCO ₃	8.00	g
Na ₂ CO ₃	22.00	g
Sulfur (powdered)	1.50	g
NH ₄ Cl	0.20	g
MgCl ₂ x 6 H ₂ O	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.05	g
Ethanol absolute	0.60	ml
Na-acetate	0.40	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	2.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.24	g
Distilled water	1000.00	ml

1. Dissolve sodium chloride and hydrogenphosphate, then sparge solution with 100% N₂ gas for 30 -45 min to make it anoxic. Add and dissolve carbonates, then dispense under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials containing already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. Sterilize medium by heating cultivation vessels in a water bath to 90 - 100°C for 1- 2 hours on each of 3 successive days. After sterilization add ammonium chloride, magnesium chloride, trace elements, yeast extract, ethanol, acetate, thiosulfate, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. The stock solutions of thiosulfate and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 9.5.

2. After inoculation add sterile 100% H₂ gas to 0.5 bar overpressure.

For DSM 19089: Replace ethanol with 1.10 g/l sodium butyrate.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
FeSO ₄ x 7 H ₂ O	2.20	g
ZnSO ₄ x 7 H ₂ O	0.10	g
MnCl ₂ x 4 H ₂ O	0.03	g
H ₃ BO ₃	0.03	g
CoCl ₂ x 6 H ₂ O	0.20	g
CuCl ₂ x 2 H ₂ O	0.03	g
NiCl ₂ x 6 H ₂ O	0.03	g

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Na ₂ MoO ₄ x 2 H ₂ O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	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