

377a: PALAEOCOCCUS MEDIUM

NaCl	30.00	g
MgSO ₄ x 7 H ₂ O	3.50	g
MgCl ₂ x 6 H ₂ O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H ₃ BO ₃	15.00	mg
SrCl ₂ x 6 H ₂ O (0.1% w/v)	7.00	ml
(NH ₄) ₂ SO ₄	10.00	mg
Citric acid (0.1% w/v)	5.00	ml
KI (0.01% w/v)	0.50	ml
CaCl ₂ x 2 H ₂ O	0.75	g
KH ₂ PO ₄	0.50	g
NiCl ₂ x 6 H ₂ O (0.1% w/v)	2.00	ml
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	30.00	g
Peptone (BD Bacto)	5.00	g
Yeast extract (OXOID)	1.00	g
Wolin's vitamin solution (10x)	1.00	ml
Neutralized sulfide solution 3% (w/v)	15.00	ml
Distilled water	960.00	ml

Dissolve ingredients except sulfur, peptone, yeast extract, vitamins, and sulfide, adjust the pH to 6.5 and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic.

Distribute medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that already contain the appropriate amount of sulfur. Sterilize the medium by autoclaving at **110°C** for 20 min. After sterilization add peptone, yeast extract, and vitamins from sterile stock solutions prepared under 100% N₂ gas atmosphere and sulfide from a neutralized stock solution. Vitamins should be sterilized by filtration.

For DSM 104102: Add 2 bar overpressure of sterile 80% H₂ and 20% CO₂ gas mixture after inoculation.

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

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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

Neutralized sulfide solution 3% (w/v) (from medium 28)

Na ₂ S x 9 H ₂ O	3.00	g
Distilled water	100.00	ml

The sulfide solution is prepared in a 250 ml screw-capped bottle with a butyl rubber septum and a magnetic stirrer. The solution is bubbled with nitrogen gas, closed and autoclaved for 15 min. at 121°C. After cooling to room temperature the pH is adjusted to about 7.0 by adding of sterile 2 M H₂SO₄ drop-wise with a syringe without opening the bottle.