

829: DESULFUROBACTERIUM MEDIUM

Sea Salt (SIGMA)	30.00	g
NH ₄ Cl	1.00	g
KH ₂ PO ₄	0.35	g
MES [2-(N-morpholino) ethane sulfonic acid]	1.95	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Na ₂ CO ₃	0.50	g
Seven vitamins solution	1.00	ml
Growth-stimulating factors	1.00	ml
Na-dithionite solution (5% w/v)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, carbonate, vitamins, growth-stimulating factors and Na-dithionite), boil medium for 1 min, then cool to room temperature under 80% H₂ and 20% CO₂ gas atmosphere. Adjust pH to 6.0 and dispense under 80% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials which contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large head space. Autoclave at a temperature of **110°C** for 20 min! Add vitamins and growth-stimulating factors from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate and Na-dithionite from sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins and Na-dithionite are sterilized by filtration. Adjust pH of the complete medium to 6.0, if necessary.
2. After inoculation use 1 bar overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.

For DSM 16661, DSM 21157: Omit growth-stimulating factors and pressurize vials after inoculation to 2 bar with sterile 80% H₂ and 20% CO₂ gas mixture.

Growth-stimulating factors (from medium 829)

Isobutyric acid	5.00	g
Valeric acid	5.00	g
2-Methylbutyric acid	5.00	g
3-Methylbutyric acid	5.00	g
Caproic acid	2.00	g
Succinic acid	6.00	g
Distilled water	1000.00	ml

Dissolve ingredients, adjust pH to 9.0 with NaOH, then autoclave under 100% N₂ gas.

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Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H ₂ O	200.00	mg
Distilled water	1000.00	ml

Na-dithionite solution (5% w/v) (from medium 829)

NaHCO ₃	50.00	g
Na ₂ S ₂ O ₄	50.00	g
Distilled water	1000.00	ml

Dissolve NaHCO₃ in water and make the solution anoxic by sparging with 80% N₂ and 20% CO₂ gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.