829: DESULFUROBACTERIUM MEDIUM

This recipe contains strain-specific modifications for *Nautilia abyssi* DSM 21157 *

Final pH: 6.0
Final volume: 1005 ml

Sea Salt (SIGMA, must not show signs of caking)	30.00	g
NH_4Cl	1.00	g
KH_2PO_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_2CO_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% H_2 and 20% CO_2 gas atmosphere. Adjust pH to 6.0 and dispense under 80% H_2 and 20% CO_2 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% N_2 gas and carbonate and Na-dithionite from sterile anoxic stock solution prepared under 80% N_2 and 20% CO_2 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% H_2 and 20% CO_2 gas mixture.

* Omit growth-stimulating factors and pressurize vials after inoculation to 2 bar with sterile 80% H_2 and 20% CO_2 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

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Dissolve ingredients, adjust pH to 9.0 with NaOH, then autoclave under 100% N_2 gas.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	36.00	mg
Distilled water	990.00	ml

First dissolve $FeCl_2$ 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
$Na_2SeO_3 \times 5\ H_2O$	3.00	mg
$Na_2WO_4 \times 2\ H_2O$	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 $\times 2\ H_2O$	200.00	mg
Distilled water	1000.00	ml

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

NaOH (1 N)	0.50	ml
$Na_2S_2O_4$	0.50	g
Distilled water	9.50	ml

Add 1 N NaOH to distilled water and make the solution anoxic by sparging with 100% N_2 gas. Then dissolve the Na-dithionite and filter-sterilize the solution into anoxic Hungate tubes. The final pH of the solution should be between 9 and 10. Store the prepared

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solution in a dark and cool place. Prepare only small amounts of stock solution, as Na -dithionite decomposes rapidly.

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