

792c: NAUTILIA NITRATIREDUCTENS MEDIUM

Solution A	958.00	ml
Solution B	10.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	15.00	ml

1. Sparge solution A with 80% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials (e.g., 20 ml medium in 100 ml bottles) and autoclave. Solutions B and C are autoclaved separately under 80% N₂ and 20% CO₂ gas mixture. Solution D is prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solution E is autoclaved under 100% N₂ gas atmosphere. To complete the medium appropriate amounts of solutions B to E are added to solution A. Adjust pH of the complete medium to 6.5, if necessary.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.
3. Note: Solution A has to be prepared freshly. It cannot be stored for a long period of time.

Solution A

NaCl	20.00	g
MgSO ₄ x 7 H ₂ O	3.50	g
MgCl ₂ x 6 H ₂ O	2.75	g
CaCl ₂ x 2 H ₂ O	0.75	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
KI (0.01% w/v)	0.50	ml
Wolfe's mineral elixir	1.00	ml
Distilled water	950.00	ml

Solution B

KNO ₃	1.00	g
KH ₂ PO ₄	0.50	g
Distilled water	10.00	ml

Solution C

Na ₂ CO ₃	1.00	g
Distilled water	20.00	ml

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Solution D

Wolin's vitamin solution (10x)	1.00	ml
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Solution E

Na ₂ S x 9 H ₂ O	0.45	g
Distilled water	15.00	ml

Wolfe's mineral elixir (from medium 792)

MgSO ₄ x 7 H ₂ O	30.00	g
MnSO ₄ x H ₂ O	5.00	g
NaCl	10.00	g
FeSO ₄ x 7 H ₂ O	1.00	g
CoCl ₂ x 6 H ₂ O	1.80	g
CaCl ₂ x 2 H ₂ O	1.00	g
ZnSO ₄ x 7 H ₂ O	1.80	g
CuSO ₄ x 5 H ₂ O	0.10	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.18	g
H ₃ BO ₃	0.10	g
Na ₂ MoO ₄ x 2 H ₂ O	0.10	g
(NH ₄) ₂ Ni(SO ₄) ₂ x 6 H ₂ O	2.80	g
Na ₂ WO ₄ x 2 H ₂ O	0.10	g
Na ₂ SeO ₄	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H₂SO₄, then add and dissolve the salts.

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