

399a: ARCHAEOGLOBUS NEPTUNIUS MEDIUM

Final pH: 6.5

Final volume: 1003 ml

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	4.00	g
MgSO ₄ x 7 H ₂ O	0.14	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	18.00	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract (OXOID)	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	0.50	g
Na-pyruvate	1.00	g
Na ₂ SO ₃	0.60	g
Wolin's vitamin solution (10x)	1.00	ml
DL-Dithiothreitol	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except for carbonate, pyruvate, sulfite (Na₂SO₃), vitamins, and dithiothreitol, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into serum vials (e.g., 20 ml per 100 ml bottle), seal, and autoclave. Before use add carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Pyruvate, sulfite, vitamins, and dithiothreitol are added from sterile anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration. Adjust the pH of the complete medium to 6.5, if necessary.

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

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Distilled water	990.00	ml
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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
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	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