

829d: DESULFUROBACTERIUM INDICUM MEDIUM

Sea Salt (SIGMA)	30.00	g
NH ₄ Cl	0.30	g
KH ₂ PO ₄	0.35	g
MES [2-(N-morpholino) ethane sulfonic acid]	1.95	g
Na-acetate	0.16	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur, powdered	10.00	g
Na ₂ CO ₃	0.50	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except sulfur, carbonate, vitamins and sulfide), 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 sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are sterilized by filtration. Adjust pH of the complete medium to 6.0 - 6.5, if necessary.

2. After inoculation add 1 bar overpressure of sterile 80% H₂ and 20% CO₂ gas mixture.

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
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

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Distilled water	1000.00	ml
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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