

135b: ACETOBACTERIUM DEHALOGENANS MEDIUM

NH ₄ Cl	1.00	g
KH ₂ PO ₄	0.33	g
K ₂ HPO ₄	0.45	g
MgSO ₄ x 7 H ₂ O	0.10	g
Modified Wolin's mineral solution	20.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Na-syringate solution (0.3 M)	15.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.50	g
Distilled water	970.00	ml

1. Dissolve ingredients except for carbonate, Na-syringate solution, vitamins, and cysteine, bring to the boil and cool to room temperature under 80% N₂ and 20% CO₂ gas atmosphere. Thereafter, dispense under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Add Na-syringate solution, vitamins, and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Vitamins should be sterilized by filtration. The pH of the completed medium should be at 7.4.

2. Note: After growth has been started add an additional amount of Na-syringate to reach a final concentration of 2.00 g/l.

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
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

Na-syringate solution (0.3 M)

Syringic acid (SIGMA S6881)	60.00	g
NaOH (5 N)	50.00	ml
Distilled water	950.00	ml

Dissolve syringic acid in 800 ml distilled water, add around 50 ml 5 N NaOH, and adjust pH to about 7.0. Then add water to reach a volume of 1000 ml. Make the solution anoxic by sparging with 100% N₂ gas, distribute in anoxic serum vials and autoclave at 121°C.