

1824: HYDROGENOVIBRIO MEDIUM

NaCl	27.50	g
MgSO ₄ x 7 H ₂ O	6.78	g
MgCl ₂ x 6 H ₂ O	5.38	g
CaCl ₂ x 2 H ₂ O	1.40	g
NH ₄ Cl	1.00	g
KCl	0.72	g
K ₂ HPO ₄	0.05	g
Modified Wolin's mineral solution	1.00	ml
Na ₂ CO ₃	0.50	g
Na ₂ S ₂ O ₃ x 5 H ₂ O	1.25	g
Wolin's vitamin solution (10x)	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, thiosulfate, and vitamins, 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 or Hungate tubes to 30% of their volume and autoclave. Add vitamins and thiosulfate 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 mixture. Vitamins and thiosulfate should be sterilized by filtration. Adjust the pH of the complete medium to 6.2-6.5, if necessary.

2. After inoculation add sterile air (with a hypodermic syringe through the rubber closure) to a concentration of ca. 1 - 3% (v/v) O₂ in the vial (e.g., add 2 ml air to a Hungate-type tube of 16 ml total volume).

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