

854: HIPPEA MEDIUM

NH ₄ Cl	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
MgCl ₂ x 6 H ₂ O	0.33	g
KCl	0.33	g
KH ₂ PO ₄	0.33	g
NaCl	25.00	g
MOPS buffer (SIGMA)	3.00	g
Modified Wolin's mineral solution	10.00	ml
Yeast extract	0.10	g
Na-acetate	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Sulfur (powdered)	10.00	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

Dissolve ingredients (except sulfur, vitamins, and sulfide), adjust pH to 5.5 - 6.0, and sparge the medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense the medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already an appropriate amount of sulfur powder. Autoclave 20 min at **110°C**. Prior to use add vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas. Vitamins are sterilized by filtration. Adjust the pH of the complete medium to 6.0 - 6.2, if necessary.

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