

494. ACETOHALOBIUM MEDIUM

NaCl	150.00	g
KCl	0.33	g
NH ₄ Cl	0.33	g
KH ₂ PO ₄	0.33	g
CaCl ₂ x 2 H ₂ O	0.33	g
MgCl ₂ x 6 H ₂ O	4.00	g
Trace element solution (see medium 141)	10.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Na ₂ CO ₃	2.00	g
Yeast extract	0.05	g
Trimethylamine-HCl	2.40	g
Vitamin solution (see medium 141)	10.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonate, yeast extract, trimethylamine, vitamins and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add yeast extract, trimethylamine, vitamins (sterilized by filtration) 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 atmosphere. Prior to use check pH of complete medium and adjust to 7.6 – 8.0 with a sterile anoxic stock solution of Na₂CO₃ (5% w/v), if necessary.