

1237. TEPIDIMICROBIUM MEDIUM

KH ₂ PO ₄	0.49	g
Na ₂ HPO ₄	1.19	g
NaCl	25.00	g
NH ₄ Cl	0.30	g
MgCl ₂ x 6 H ₂ O	0.10	g
CaCl ₂ x 2 H ₂ O	0.01	g
Trace elements solution (see medium 141)	10.00	ml
Tryptone (BD Bacto)	2.00	g
Proteose peptone (BD Difco)	2.00	g
Yeast extract (OXOID)	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
FeCl ₂ solution (0.1% w/v in 0.2 N HCl)	1.50	ml
NaHCO ₃	5.00	g
D-Glucose	5.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferrous chloride, bicarbonate, glucose, vitamins and reducing agents), adjust pH to 7.5 with NaOH and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, ferrous chloride, sulfide and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas and bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Solutions of ferrous chloride and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 8.5, if necessary.