1636. TEPIDIBACILLUS MEDIUM

NaCl	10.00	g
KH_2PO_4	0.33	g
$MgCl_2$ x 6 H_2O	0.33	g
KCl	0.33	g
NH_4Cl	0.33	g
Trace element solution (see medium 141)	1.00	ml
Yeast extract	0.10	g
$CaCl_2$ x 2 H_2O	0.33	g
Na_2CO_3	1.50	g
D-Glucose	2.00	g
Vitamin solution (see medium 141)	10.00	ml
Na_2S x 9 H_2O	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except calcium chloride, bicarbonate, glucose, vitamins and sulfide), then sparge medium with 80% N_2 and 20% CO_2 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. After autoclaving add calcium chloride, glucose, vitamins and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. The vitamin solution should be sterilized by filtration. Adjust pH of the complete medium to 7.4, if necessary.