1237. TEPIDIMICROBIUM MEDIUM

KH_2PO_4	0.49	g
Na_2HPO_4	1.19	g
NaCl	25.00	g
NH_4Cl	0.30	g
$MgCl_2$ x 6 H_2O	0.10	g
$CaCl_2$ x 2 H_2O	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
Na_2CO_3	2.50	g
D-Glucose	5.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except ferrous chloride, carbonate, glucose, vitamins, cysteine and sulfide), adjust pH to 7.5 with NaOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under 100% N_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add glucose, vitamins, ferrous chloride, cysteine and sulfide form sterile anoxic stock solutions prepared under 100% N_2 gas and carbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Solutions of ferrous chloride and vitamins should be sterilized by filtration. Adjust pH of the complete medium to 8.5, if necessary.