Microorganisms



lain sol. 1665		
NaCl	20.00	g
$MgCl_2 \ge 6 H_2O$	6.00	g
Na ₂ SO ₄	3.00	g
$CaCl_2 \times 2 H_2O$	0.20	g
KCI	0.50	g
NH ₄ Cl	0.30	g
Wolfe's mineral elixir	2.00	ml
HEPES (SIGMA)	2.40	g
Na ₂ -fumarate	1.00	g
Yeast extract (OXOID)	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
K-phosphate buffer (0.5 M, pH 7.0)	2.00	ml
NaHCO ₃	1.00	g
FeCl ₂ x 4 H ₂ O (0.2% w/v in 0.02 N HCl)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphate buffer, bicarbonate, ferrous chloride, vitamins and cysteine), adjust pH to 7.2 with NaOH and sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add phosphate buffer, ferrous chloride, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N₂ gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.2 - 7.5, if necessary.