



Main sol. 769

Sea Salt (SIGMA)	12.00	g
NH ₄ NO ₃	0.10	g
Yeast extract (BD Bacto)	1.00	g
Trypticase peptone (BD BBL)	0.40	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	1.00	g
Na-phosphate buffer (0.2 M, pH 6.5)	20.00	ml
Lactose	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.30	g
Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	980.00	ml

1. Dissolve ingredients (except bicarbonate, phosphate-buffer, lactose, vitamins, cysteine 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. After sterilization add phosphate-buffer, lactose, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas and bicarbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are prepared under N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 6.5, if necessary.

2. Note: Sea salts can be replaced by 300.0 ml/l of filtered aged sea water.