

769: CLOSTRIDIUM VINCENTII MEDIUM

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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml