

502: PSEUDOCLOSTRIDIUM MEDIUM

NaCl	1.20	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.30	g
CaCl ₂ x 2 H ₂ O	0.15	g
NH ₄ Cl	0.27	g
KH ₂ PO ₄	0.21	g
Na ₂ SO ₄	0.10	g
Trace element solution SL-10	1.00	ml
Inulin	5.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract	0.03	g
Casamino acids (BD Bacto)	0.03	g
Na ₂ CO ₃	1.50	g
Na ₂ HPO ₄	2.66	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.15	g
Distilled water	1000.00	ml

Dissolve ingredients (except yeast extract, Casamino acids, vitamins, carbonate, hydrogenphosphate and sulfide), bring medium to the boil, then cool to room temperature under 80% N₂ and 20% CO₂ gas mixture. Dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add yeast extract, Casamino acids, hydrogenphosphate and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 7.0, if necessary.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

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