318b: ANAEROTIGNUM NEOPROPIONICUM MEDIUM

KH_2PO_4	0.30	g
NaCl	0.60	g
$MgCl_2 \times 6\ H_2O$	0.10	g
$CaCl_2 \times 2\ H_2O$	0.08	g
Trace element solution	10.00	ml
NH_4Cl	1.00	g
Yeast extract (OXOID)	0.50	g
Trypticase peptone (BD BBL)	0.50	g
Sodium resazurin (0.1% w/v)	0.50	ml
$KHCO_3$	4.00	g
Ethanol	1.30	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl $\times H_2O$	0.30	g
$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, ethanol, vitamins, cysteine and sulfide), then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense under 80% N_2 and 20% CO_2 gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ethanol, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N_2 gas. Vitamins are prepared under 100% N_2 gas and sterilized by filtration. Adjust pH of complete medium to 7.0 - 7.2, if necessary.

Trace element solution (from medium 318)

Nitrilotriacetic acid (NTA)	12.80	g
$FeCl_2 \times 4\ H_2O$	1.00	g
$MnCl_2 \times 4\ H_2O$	0.10	g
$CoCl_2 \times 6\ H_2O$	0.03	g
$CaCl_2 \times 2\ H_2O$	0.10	g
$ZnCl_2$	0.10	g
$CuCl_2$	0.02	g
H_3BO_3	0.01	g
$Na_2MoO_4 \times 2\ H_2O$	0.03	g
$NiCl_2 \times 6\ H_2O$	0.10	g
NaCl	1.00	g
$Na_2SeO_3 \times 5\ H_2O$	0.03	g
$Na_2WO_4 \times 2\ H_2O$	0.04	g
Distilled water	1000.00	ml

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First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and 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

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