

194b: OXALOPHAGUS MEDIUM

Solution A	962.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml

Dissolve ingredients of solution A and adjust pH to 6.8, then sparge solution with 80% N₂ and 20% CO₂ gas mixture for at least 30 - 45 min to make it anoxic. Distribute solution under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solution D is autoclaved under 100% N₂ gas. Solutions B to D are added to the sterile, cooled solution A in appropriate amounts in the sequence as indicated. Final pH of the medium should be 6.8 - 7.0.

Solution A

Na ₂ SO ₄	3.00	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
NaCl	1.00	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium acetate	0.20	g
Ammonium oxalate	3.00	g
Yeast extract	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	960.00	ml

Solution B

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml
---------------------------------------	------	----

Solution D

Na ₂ S x 9 H ₂ O	0.40	g
Distilled water	10.00	ml

194b: OXALOPHAGUS MEDIUM

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.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ O	4.00	mg
Distilled water	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 B12	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml