

386a: THIOVIBRIO MEDIUM

Solution A	967.00	ml
Solution B	30.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Sulfur (powdered)	10.00	g

Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas mixture. Solutions C and D are prepared under a 100% N₂ gas atmosphere and sterilized by filtration. To complete the medium solutions B to D are added to the sterile solution A in the sequence as indicated. Before inoculation, appropriate amounts of sterile sulfur are added to the vials with the medium. Sulfur is sterilized by steaming for 3 hours on each of 3 successive days (see medium 35) and added aseptically to the sterile medium while retaining anoxic conditions. The final pH of the medium should be at 7.0.

Solution A

KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	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
Amorphous Fe(OH)₃	25.00	ml
Distilled water	940.00	ml

Solution B (from medium 386)

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C

Seven vitamins solution	1.00	ml
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Solution D

DL-Dithiothreitol	0.30	g
Distilled water	10.00	ml

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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

Amorphous Fe(OH)₃ (from medium 1210)

FeCl ₃ x 6 H ₂ O (60.00 g/l)	320.00	ml
NaOH (10% w/v), adjust if required	80.00	ml

Slowly titrate 320 ml of a FeCl₃ x 6 H₂O stock solution (60.00 g/l) with 10% (w/v) NaOH to pH 8.0-8.5 under agitation (use magnetic stirrer). Total amount of added NaOH approx. 80 - 100 ml. The precipitated Fe(OH)₃ should be stored at room temperature overnight with surface covered with water. Thereafter, centrifuge at 2000 rpm for 5 min and discard the supernatant. Wash several times with distilled water. Resuspend the pellet in medium as described above. For storage autoclave under 100% N₂ atmosphere.

Seven vitamins solution (from medium 503)

Vitamin B ₁₂	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg
Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H ₂ O	200.00	mg
Distilled water	1000.00	ml