

505: FORMIVIBRIO MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic, distribute under 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. Solutions C and E are autoclaved under 100% N₂ gas. Solution D is prepared under 100% N₂ gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Adjust pH of complete medium to 7.5 - 7.8 with a sterile anoxic stock solution of Na₂CO₃, if necessary.

For <u>DSM 6139</u>: Use instead of citrate 10 mM sodium cinnamate (or 0.2% sucrose or 0.2% sodium pyruvate) as substrate and supplement the medium with 0.05 g/l of yeast extract. Sterilize substrates separately. Adjust pH of the complete medium to 7.7 - 7.9.

Solution A		
KH ₂ PO ₄	1.40	g
NH ₄ Cl	0.50	g
$MgCl_2 \times 6 H_2O$	0.20	g
$CaCl_2 \times 2 H_2O$	0.15	g
Sodium resazurin (0.1% w/v)	0.50	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Distilled water	940.00	ml
Solution B		
Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml
Solution C		
Trisodium citrate x 2 H_2O	3.00	g
Distilled water	20.00	ml
Solution D		
Wolin's vitamin solution (10x)	1.00	ml

Microorganisms

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Solution E

$Na_2S \times 9 H_2O$	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCI (25%)	10.00	ml
$FeCl_2 \times 4 H_2O$	1.50	g
ZnCl ₂	70.00	mg
$MnCl_2 \times 4 H_2O$	100.00	mg
H ₃ BO ₃	6.00	mg
$CoCl_2 \times 6 H_2O$	190.00	mg
$CuCl_2 \times 2 H_2O$	2.00	mg
$NiCl_2 \times 6 H_2O$	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl_2 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
$Na_2SeO_3 \times 5 H_2O$ 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 HCI	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