

311c: SPOROMUSA ACIDOVORANS MEDIUM

This recipe contains strain-specific modifications for *Acetone nema longum* DSM 6540 *

Final pH: 6.5 - 7.0

Final volume: 1005 ml

NH ₄ Cl	0.50	g
MgSO ₄ x 7 H ₂ O	0.50	g
CaCl ₂ x 2 H ₂ O	0.25	g
NaCl	2.25	g
FeSO ₄ x 7 H ₂ O (0.1% w/v in 0.1 N H ₂ SO ₄)	2.00	ml
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	2.00	g
Casitone	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
K ₂ HPO ₄	0.35	g
KH ₂ PO ₄	0.23	g
Na ₂ CO ₃	1.00	g
D-Fructose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H₂O	0.30	g
Na₂S x 9 H₂O	0.30	g
DL-Dithiothreitol	0.15	g
D-Glucose	2.00	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphates, carbonate, fructose, vitamins, cysteine and sulfide) and sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add phosphates, fructose, vitamins, cysteine and sulfide to the medium 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. Stock solutions of fructose and vitamins should be sterilized by filtration. Adjust pH of complete medium to pH 6.5 - 7.0, if necessary.

* Replace fructose with 2.00 g/l D-glucose added to the autoclaved medium from a filter-sterilized anoxic stock solution. Cysteine and sulfide must be replaced by 0.15 g/l DL-dithiothreitol (DTT) added from an anoxic stock solution sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g

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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 B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml