

### 311c: SPOROMUSA ACIDOVORANS MEDIUM

This recipe contains strain-specific modifications for *Acetonea longum* DSM 6540 \*

Final pH: 6.5 - 7.0

Final volume: 1005 ml

NH <sub>4</sub> Cl	0.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.25	g
NaCl	2.25	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O (0.1% w/v in 0.1 N H <sub>2</sub> SO <sub>4</sub> )	2.00	ml
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	2.00	g
Casitone	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
K <sub>2</sub> HPO <sub>4</sub>	0.35	g
KH <sub>2</sub> PO <sub>4</sub>	0.23	g
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
<del>D-Fructose</del>	<del>5.00</del>	<del>g</del>
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
<del>L-Cysteine HCl x H<sub>2</sub>O</del>	<del>0.30</del>	<del>g</del>
<del>Na<sub>2</sub>S x 9 H<sub>2</sub>O</del>	<del>0.30</del>	<del>g</del>
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% N<sub>2</sub> and 20% CO<sub>2</sub> 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% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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 <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g

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ZnCl <sub>2</sub>	70.00	mg
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> 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 <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml