

## 195c: DESULFOBACTER SP. MEDIUM (LACTATE)

This recipe contains strain-specific modifications for *Nitrosopumilus zosteræ* DSM 11974 \*

Final pH: 7.1 - 7.4

Final volume: 1003 ml

<b>Solution A</b>	952.00	ml
<b>Solution B</b>	30.00	ml
<b>Solution C</b>	10.00	ml
<b>Solution D</b>	1.00	ml
<b>Solution E</b>	10.00	ml
<b>Seven vitamins solution</b>	1.00	ml

1. Solution A is sparged with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclaved. Solutions C and E are autoclaved separately under 100% N<sub>2</sub> gas. Solution B is autoclaved under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solution D is prepared under 100% N<sub>2</sub> gas atmosphere 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. Final pH of the medium should be 7.1 - 7.4.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth at the beginning. For transfers use 5 - 10% (v/v) inoculum.

\* Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

### Solution A

Na <sub>2</sub> SO <sub>4</sub>	3.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.30	g
NaCl	21.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	3.00	g
KCl	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

### Solution B

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
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## 195c: DESULFOBACTER SP. MEDIUM (LACTATE)

Distilled water	30.00	ml
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### Solution C

Na-L-lactate	2.50	g
Distilled water	10.00	ml

### Solution D

<b>Wolin's vitamin solution (10x)</b>	1.00	ml
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### Solution E

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	10.00	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

### 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
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.

### 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

## 195c: DESULFOBACTER SP. MEDIUM (LACTATE)

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

## **Seven vitamins solution\*** (from medium 503)

Vitamin B <sub>12</sub>	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 <sub>2</sub> O	200.00	mg
Distilled water	1000.00	ml