

## 194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

This recipe contains strain-specific modifications for *Acetobacterium sp.* DSM 5193 \*

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

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 into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C and E are autoclaved under 100% N<sub>2</sub> gas. Solution D is prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Solutions B to E are added to the sterile, cooled solution A in appropriate amounts 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 of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

\* Supplement medium with 0.50 g/l yeast extract and replace Na-propionate with 0.90 g/l Na-methoxyacetate added after autoclaving from a sterile anoxic stock solution prepared under N<sub>2</sub>.

### 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	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	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
<b>Yeast extract</b>	<b>0.50</b>	<b>g</b>
Distilled water	950.00	ml

## 194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

### Solution B

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Distilled water	30.00	ml

### Solution C

<del>Na propionate</del>	<del>1.50</del>	<del>g</del>
Acetic acid, 2-methoxy-, sodium salt (1:1)	0.90	g
Distilled water	10.00	ml

### Solution D

Wolin's vitamin solution (10x)	1.00	ml
--------------------------------	------	----

### Solution E

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

### 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

### 194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

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