

### 383: DESULFOBACTERIUM MEDIUM

This recipe contains strain-specific modifications for *Desulfobacula phenolica* DSM 3384 \*

Final pH: 7.0 - 7.2

Final volume: 1003 ml

<b>Solution A</b>	952.00	ml
<b>Solution B</b>	20.00	ml
<b>Solution C</b>	20.00	ml
<b>Solution D</b>	1.00	ml
<b>Solution E</b>	10.00	ml
<b>Na-dithionite solution (5% w/v)</b>	0.50	ml
<b>Clarified rumen fluid</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 in anoxic cultivation vials and autoclaved under the same gas atmosphere. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C and D are prepared under 100% N<sub>2</sub> gas and filter-sterilized. Solution E is autoclaved under 100% N<sub>2</sub> gas. 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 7.0 - 7.2.

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% inoculum. Incubate all strains in the dark.

\* The vitamin solution of medium 120 is replaced with 1.00 ml/l of seven vitamins solution of medium 503. Replace pyruvate with 0.40 g/l Na-benzoate and add 10.00 ml/l clarified rumen fluid (see medium 1310) as growth supplement. Initiation of growth requires addition of freshly prepared sodium dithionite.

#### 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

## 383: DESULFOBACTERIUM MEDIUM

### Solution B

Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Distilled water	20.00	ml

### Solution C

<del>Na-pyruvate</del>	<del>2.50</del>	<del>g</del>
Na-benzoate	0.40	g
Distilled water	20.00	ml

### Solution D

<del>Wolin's vitamin solution (10x)</del>	<del>1.00</del>	<del>ml</del>
Seven vitamins solution	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

### 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
--------	-------	----

## 383: DESULFOBACTERIUM MEDIUM

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

### Na-dithionite solution (5% w/v)\* (from medium 829)

NaOH (1 N)	1.00	ml
Na <sub>2</sub> S <sub>2</sub> O <sub>4</sub>	0.50	g
Distilled water	9.00	ml

Add 1 N NaOH to distilled water and make the solution anoxic by sparging with 100% N<sub>2</sub> gas. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in a dark and cool place. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.

### 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

### Clarified rumen fluid\* (from medium 1310)

Rumen fluid from cow or sheep (obtained from fistulated animals or abattoir refuse) is filtered through muslin, autoclaved at 121°C for 15 min and then centrifuged at 27,000 g for 20 min. The supernatant is made anoxic by sparging with 100% N<sub>2</sub> gas for 15 min, dispensed under same gas atmosphere into anoxic serum vials to 30% of volume and then stored frozen at -20°C.