

## 916: DESULFOFABA HANSENII MEDIUM

<b>Solution A</b>	912.00	ml
<b>Solution B</b>	50.00	ml
<b>Solution C</b>	25.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	1.00	ml
<b>Solution F</b>	10.00	ml

1. Sparge solution A with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solutions B and D are autoclaved under 100% N<sub>2</sub> gas atmosphere. Solution C is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions E and F are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B to F are added to the sterile solution A in the sequence as indicated. The pH of the complete 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 at the beginning.

### Solution A

NaCl	20.00	g
KCl	0.67	g
NH <sub>4</sub> Cl	0.10	g
KH <sub>2</sub> PO <sub>4</sub>	0.01	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.02	g
Na <sub>2</sub> SO <sub>4</sub>	1.42	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	910.00	ml

### Solution B

MgCl <sub>2</sub> x 6 H <sub>2</sub> O	10.60	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	1.52	g
Distilled water	50.00	ml

### Solution C

Na <sub>2</sub> CO <sub>3</sub>	1.25	g
Distilled water	25.00	ml

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### Solution D

Na-propionate	0.96	g
Distilled water	10.00	ml

### Solution E

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

FeCl <sub>2</sub> x 4 H <sub>2</sub> O	0.52	g
Distilled water	10.00	ml

Adjust to pH 2 with HCl.

### 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
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg

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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