## **Microorganisms**



### 386: DESULFOLUTIVIBRIO MEDIUM

Solution A	942.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	13.00	ml

- 1. Solution A is sparged 80%  $N_2$  and 20%  $CO_2$  gas mixture for 30 45 min to make it anoxic, then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80%  $N_2$  and 20%  $CO_2$  gas mixture. Solutions C and F are autoclaved under 100%  $N_2$  gas. Solutions D and E are prepared under 100%  $N_2$  gas atmosphere and sterilized by filtration. To complete the medium appropriate amounts of the solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 7.1 7.4.
- 2. Note: When growth has started feed culture again with same amount of pyrosulfite. After a further two days repeat feeding once more.

For <u>DSM 7269</u>: Replace pyrosulfite with 2.50 g/l  $Na_2S_2O_3 \times 5 H_2O$  added to the medium after autoclaving from a sterile anoxic stock solution.

Sol	lutio	n	Α
K	H <sub>a</sub> PO		

KH <sub>2</sub> PO <sub>4</sub>	0.20	g
NH <sub>4</sub> Cl	0.25	g
NaCl	1.00	g
$MgCl_2 \times 6 H_2O$	0.40	g
KCI	0.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

### **Solution B**

$Na_2CO_3$	1.50	g
Distilled water	30.00	ml

#### **Solution C**

Na-acetate x 3 H <sub>2</sub> O	0.30	g
Distilled water	10.00	ml

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Solution D Seven vitamins solution	1.00	ml
<b>Solution E</b> $Na_2S_2O_5$ (sodium pyrosulfite) Distilled water	0.95 10.00	g ml
Adjust pH to 7.5-8.0 with NaOH.		
Solution F Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.40	g
Distilled water	13.00	ml
Trace element solution SL-10 (from medium	n 320)	
HCI (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
$MnCl_2 \times 4 H_2O$	100.00 6.00	mg
$H_3BO_3$ $CoCl_2 \times 6 H_2O$	190.00	mg mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
$NiCl_2 \times 6 H_2O$	24.00	mg
$Na_2MoO_4 \times 2 H_2O$	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
$Na_2SeO_3 \times 5 H_2O$	3.00	mg
$Na_2WO_4 \times 2 H_2O$	4.00	mg
Distilled water	1000.00	ml

### Seven vitamins solution (from medium 503)

CTCII TICAIIIII DOIACIOII (II o III		
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

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mg

ml

Thiamine-HCl x 2  $H_2O$  200.00 Distilled water 1000.00