## **Microorganisms**



#### 142: THIOMICROSPIRA PELOPHILA MEDIUM

Final pH: 7.2

Final volume: 1005 ml

NaCl	25.00	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4 \times 7 H_2O$	1.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.42	g
Trace element solution (Vishniac & Santer, 1957)	0.20	ml
Bromothymol blue (0.1% w/v)	4.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.50	g
$Na_2S_2O_3 \times 5 H_2O$	5.00	g
Seven vitamins solution	1.00	ml
Distilled water 1	00.00	ml

- 1. Dissolve ingredients (except hydrogenphosphate, thiosulfate and vitamins), adjust pH to 7.2 and autoclave.  $K_2HPO_4$  and  $Na_2S_2O_3$  are autoclaved separately each in 10% of the final volume. Filter sterilize the vitamins solution. Adjust pH of the complete medium to 7.2 with sterile 0.4% (w/v)  $Na_2CO_3$  solution. Acidification of the medium during growth causes the pH indicator bromothymol blue to turn from blue to yellow.
- 2. Note: Growth of most Thiomicrospira strains is more reliable if the medium is prepared under a  $80\% N_2$  and  $20\% CO_2$  gas atmosphere to make it anoxic and then filled under air atmosphere into Hungate-type tubes (5 ml per vial). The pH is adjusted with a sterile stock solution of  $Na_2CO_3$  (5% w/v) after autoclaving.

### Trace element solution (Vishniac & Santer, 1957) (from medium 69)

Na <sub>2</sub> -EDTA	50.00	g
$ZnSO_4 \times 7 H_2O$	22.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	5.54	g
$MnCl_2 \times 4 H_2O$	5.06	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	5.00	g
$(NH_4)_6Mo_7O_{24} \times 4 H_2O$	1.10	g
$CuSO_4 \times 5 H_2O$	1.57	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	1.61	g
Distilled water	1000.00	ml

Dissolve EDTA in distilled water, adjust pH to 7 using 2 N KOH, then add remaining compounds. Adjust pH of final solution to 6.0 with KOH.

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

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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