142: THIOMICROSPIRA PELOPHILA MEDIUM

NaCl	25.00	g
$(NH_4)_2SO_4$	1.00	g
$MgSO_4$ x 7 H_2O	1.50	g
$CaCl_2$ x 2 H_2O	0.42	g
Trace element solution (Vishniac and Santer, 1957)	0.20	ml
Bromothymol blue (0.1% w/v)	4.00	ml
K_2HPO_4	0.50	g
$Na_2S_2O_3$ x 5 H_2O	5.00	g
Seven vitamins solution	1.00	ml
Distilled water	1000.00	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 and Santer, 1957) (from medium 69)

Na_2-EDTA	50.00	g
$ZnSO_4$ x 7 H_2O	22.00	g
$CaCl_2$ x 2 H_2O	5.54	g
$MnCl_2$ x 4 H_2O	5.06	g
$FeSO_4$ x 7 H_2O	5.00	g
$(NH_4)_6Mo_7O_{24}$ x 4 H_2O	1.10	g
$CuSO_4$ x 5 H_2O	1.57	g
$CoCl_2$ x 6 H_2O	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)

Vitamin B_{12}	100.00	mg
p-Aminobenzoic acid	80.00	mg
D-(+)-biotin	20.00	mg

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Nicotinic acid	200.00	mg
Calcium pantothenate	100.00	mg
Pyridoxine hydrochloride	300.00	mg
Thiamine-HCl x 2 H_2O	200.00	mg
Distilled water	1000.00	ml