# 142: THIOMICROSPIRA PELOPHILA MEDIUM

Final pH: 7.2
Final volume: 1005 ml

| | | |
|---|---|---|
| NaCl | 25.00 | g |
| $(NH_4)_2SO_4$ | 1.00 | g |
| $MgSO_4$ x 7 $H_2O$ | 1.50 | g |
| $CaCl_2$ x 2 $H_2O$ | 0.42 | g |
| **Trace element solution (Vishniac & 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 & 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)

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| | | |
|---|---|---|
| Vitamin $B_{12}$ | 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 $H_2O$ | 200.00 | mg |
| Distilled water | 1000.00 | ml |