

## 142: THIOMICROSPIRA PELOPHILA MEDIUM

This recipe contains strain-specific modifications for *Hydrogenovibrio crunogenus* DSM 12346 \*

Final pH: \* 7.3 - 7.6

Final volume: 1005 ml

NaCl	25.00	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	1.00	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	1.50	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.42	g
<b>Trace element solution (Vishniac &amp; Santer, 1957)</b>	0.20	ml
Bromothymol blue (0.1% w/v)	4.00	ml
K <sub>2</sub> HPO <sub>4</sub>	0.50	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	5.00	g
<del>Seven vitamins solution</del>	<del>1.00</del>	<del>ml</del>
Distilled water	1000.00	ml

1. Dissolve ingredients (except hydrogenphosphate, thiosulfate and vitamins), adjust pH to 7.2 and autoclave. K<sub>2</sub>HPO<sub>4</sub> and Na<sub>2</sub>S<sub>2</sub>O<sub>3</sub> 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<sub>2</sub>CO<sub>3</sub> 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<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub>CO<sub>3</sub> (5% w/v) after autoclaving.

\* Omit vitamins and adjust pH of complete medium to 7.3 - 7.6.

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

Na <sub>2</sub> -EDTA	50.00	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	22.00	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	5.54	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	5.06	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	5.00	g
(NH <sub>4</sub> ) <sub>6</sub> Mo <sub>7</sub> O <sub>24</sub> x 4 H <sub>2</sub> O	1.10	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	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.

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### Seven vitamins solution (from medium 503)

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