

## 142: THIOMICROSPIRA PELOPHILA MEDIUM

This recipe contains strain-specific modifications for *Hydrogenovibrio thermophilus* DSM 25194 \*

Final pH: \* 8.0

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 8.0.

### 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 |