

142: THIOMICROSPIRA PELOPHILA MEDIUM

This recipe contains strain-specific modifications for *Hydrogenovibrio sp.* DSM 28203 *

Final pH: * 7.3 - 7.6

Final volume: 1005 ml

| | | |
|--|-----------------|---------------|
| NaCl | 25.00 | g |
| (NH ₄) ₂ SO ₄ | 1.00 | g |
| MgSO ₄ x 7 H ₂ O | 1.50 | g |
| CaCl ₂ x 2 H ₂ O | 0.42 | g |
| Trace element solution (Vishniac & Santer, 1957) | 0.20 | ml |
| Bromothymol blue (0.1% w/v) | 4.00 | ml |
| K ₂ HPO ₄ | 0.50 | g |
| Na ₂ S ₂ O ₃ x 5 H ₂ O | 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. K₂HPO₄ and Na₂S₂O₃ 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) Na₂CO₃ 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% N₂ and 20% CO₂ 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 Na₂CO₃ (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 ₂ -EDTA | 50.00 | g |
| ZnSO ₄ x 7 H ₂ O | 22.00 | g |
| CaCl ₂ x 2 H ₂ O | 5.54 | g |
| MnCl ₂ x 4 H ₂ O | 5.06 | g |
| FeSO ₄ x 7 H ₂ O | 5.00 | g |
| (NH ₄) ₆ Mo ₇ O ₂₄ x 4 H ₂ O | 1.10 | g |
| CuSO ₄ x 5 H ₂ O | 1.57 | g |
| CoCl ₂ x 6 H ₂ 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 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 ₂ O | 200.00 | mg |
| Distilled water | 1000.00 | ml |