

142: THIOMICROSPIRA PELOPHILA MEDIUM

This recipe contains strain-specific modifications for *Hydrogenovibrio crunogenus* DSM 12354 *

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