

1277b: CALDIMICROBIUM THIODISMUTANS MEDIUM

This recipe contains strain-specific modifications for *Dissulfurispira thermophila* DSM 110365 *

Final pH: 7.5 - 8.0

Final volume: 1007 ml

MgCl ₂ x 6 H ₂ O	0.20	g
CaCl ₂ x 2 H ₂ O	0.10	g
(NH ₄) ₂ SO ₄	0.10	g
KH ₂ PO ₄	0.10	g
KCl	0.10	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Amorphous Fe(OH)₃	45.00	ml
Na ₂ CO ₃	2.50	g
Na₂S₂O₅	0.90	g
Seven vitamins solution	1.00	ml
Na ₂ S ₂ O ₃ x 5 H ₂ O	2.50	g/l
Distilled water	960.00	ml

Dissolve ingredients (except carbonate, pyrosulfite and vitamins), then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into Hungate-type tubes or serum vials and autoclave. Add pyrosulfite from a 10% (w/v) stock solution adjusted to pH 7.5-8.0 with NaOH, made anoxic with 100% N₂ gas and sterilized by filtration. Vitamins are added from a stock solution prepared under 100% N₂ gas atmosphere and sterilized by filtration and carbonate is added from a sterile stock solution prepared under 80% N₂ and 20% CO₂ gas atmosphere. Adjust pH of complete medium to 7.5 - 8.0. Incubate without shaking.

* Replace pyrosulfite with 2.5 g/l Na₂S₂O₃ x 5 H₂O added from an anoxic stock solution sterilized by filtration.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg
H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

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First dissolve FeCl_2 in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
$\text{Na}_2\text{SeO}_3 \times 5 \text{ H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{ H}_2\text{O}$	4.00	mg
Distilled water	1000.00	ml

Amorphous $\text{Fe}(\text{OH})_3$ (from medium 1210)

$\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$ (60.00 g/l)	320.00	ml
NaOH (10% w/v), adjust if required	80.00	ml

Slowly titrate 320 ml of a $\text{FeCl}_3 \times 6 \text{ H}_2\text{O}$ stock solution (60.00 g/l) with 10% (w/v) NaOH to pH 8.0-8.5 under agitation (use magnetic stirrer). Total amount of added NaOH approx. 80 - 100 ml. The precipitated $\text{Fe}(\text{OH})_3$ should be stored at room temperature overnight with surface covered with water. Thereafter, centrifuge at 2000 rpm for 5 min and discard the supernatant. Wash several times with distilled water. Resuspend the pellet in medium as described above. For storage autoclave under 100% N_2 atmosphere.

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 $\times 2 \text{ H}_2\text{O}$	200.00	mg
Distilled water	1000.00	ml