

1163: KOSMOTOGA OLEARIA MEDIUM

This recipe contains strain-specific modifications for *Tepiditoga spiralis* DSM 105848 *

Final pH: 6.8

Final volume: 1011 ml

NaCl	30.00	g
PIPES (SIGMA)	6.70	g
KCl	0.33	g
MgCl ₂ x 2 H ₂ O	0.90	g
MgSO ₄ x 7 H ₂ O	1.40	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
KH ₂ PO ₄	0.45	g
Yeast extract	5.00	g
Modified Wolin's mineral solution	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
Maltose	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
Na-thiosulfate	1.50	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, maltose and vitamins. Sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic, then add and dissolve cysteine and adjust pH to 6.8 with NaOH. Dispense medium under 100% N₂ gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add maltose and vitamins after autoclaving from anoxic stock solutions prepared under 100% N₂ gas and sterilized by filtration.

* Supplement medium with 1.50 g/l Na-thiosulfate added to the autoclaved medium from an anoxic stock solution prepared under 100% N₂ gas and sterilized by filtration.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g

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H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml