

396: METHANOSALSUM ZHILINAE MEDIUM

Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
NaCl	40.00	g
NH ₄ Cl	1.00	g
MgCl ₂ x 6 H ₂ O	3.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
KCl	1.00	g
K ₂ HPO ₄	0.40	g
Modified Wolin's mineral solution	5.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
L-Cysteine HCl x H ₂ O	0.50	g
NaHCO ₃	3.00	g
Na ₂ CO ₃	2.00	g
Trimethylamine-HCl	2.00	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	1000.00	ml

Dissolve ingredients except cysteine, bicarbonate, carbonate, trimethylamine and sulfide, then sparge medium with 100% N₂ gas for 30 - 45 min to make it anoxic. Add cysteine and dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Add trimethylamine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas, whereas bicarbonate and carbonate are added from sterile anoxic stock solutions prepared under 80% N₂ and 20% CO₂ gas atmosphere. Adjust pH of complete medium to 9.2 - 9.4.

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