

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
Trace element solution (see medium 141)	5.00	ml
Na-resazurin solution (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.