

141c. METHANOSARCINA SP. MEDIUM (N₂/CO₂)

KCl	0.34	g
MgCl ₂ x 6 H ₂ O	4.00	g
MgSO ₄ x 7 H ₂ O	3.45	g
NH ₄ Cl	0.25	g
CaCl ₂ x 2 H ₂ O	0.14	g
K ₂ HPO ₄	0.14	g
NaCl	18.00	g
Trace element solution (see medium 141)	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O solution (0.1% w/v)	2.00	ml
Na-acetate	1.00	g
Yeast extract (OXOID)	2.00	g
Trypticase peptone (BD BBL)	2.00	g
Na-resazurin solution (0.1% w/v)	0.50	ml
NaHCO ₃	5.00	g
Trimethylammonium chloride	5.00	g
Vitamin solution (see medium 141)	10.00	ml
L-Cysteine-HCl x H ₂ O	0.50	g
Na ₂ S x 9 H ₂ O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except bicarbonate, trimethylammonium chloride, vitamins, cysteine and sulfide), sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic. Add and dissolve bicarbonate and adjust pH to 7.0, then dispense medium under 80% N₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add trimethylammonium chloride, cysteine and sulfide from sterile anoxic stock solutions autoclaved under 100% N₂ gas atmosphere. Vitamins are prepared under 100% N₂ gas atmosphere and sterilized by filtration. Adjust pH of complete medium to 7.0 – 7.5, if necessary.

For [DSM 2834](#), [DSM 3028](#), [DSM 3029](#), [DSM 3318](#), [DSM 4659](#), [DSM 6636](#), [DSM 13159](#), [DSM 15192](#), [DSM 21213](#), [DSM 21221](#), [DSM 21339](#) and [DSM 104044](#) replace trimethylammonium chloride with 5.00 ml/l methanol added to the autoclaved medium from a sterile anoxic stock solution. Adjust pH of complete medium to 6.8 – 7.2.

For [DSM 5309](#), [DSM 21301](#), [DSM 21708](#) and [DSM 24758](#) omit trimethylammonium chloride from the medium.

For [DSM 6564](#) adjust pH of complete medium to 6.8.