

141c: METHANOCOCCOIDES MEDIUM

Final pH: 7.0 - 7.4

Final volume: 1013 ml

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
Modified Wolin's mineral solution	10.00	ml
Fe(NH ₄) ₂ (SO ₄) ₂ x 6 H ₂ O (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
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO ₃	5.00	g
Trimethylammonium chloride	5.00	g
Wolin's vitamin solution (10x)	1.00	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 6.8, 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 - 7.4, if necessary.

For [DSM 2831](#): Replace trimethylammonium chloride with 5.00 g/l sucrose added from a sterile anoxic stock solution prepared under 100% N₂ gas.

For [DSM 2834](#), [DSM 3028](#), [DSM 3029](#), [DSM 3318](#), [DSM 4659](#), [DSM 6636](#), [DSM 13159](#), [DSM 21213](#), [DSM 21221](#), [DSM 21339](#), [DSM 15192](#), [DSM 104044](#), [DSM 100371](#): Replace trimethylammonium chloride with 10.00 ml/l methanol solution (50% v/v) added to the autoclaved medium from a sterile anoxic stock solution. Adjust pH of complete medium to 6.8 - 7.2.

For [DSM 4138](#): Replace trimethylammonium chloride with 5.00 g/l D-glucose added from a sterile anoxic stock solution prepared under 100% N₂ gas. Use an overpressure of 2 bar of

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80% N₂ and 20% CO₂ gas mixture.

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

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

For [DSM 11916](#): Adjust pH to 6.6.

For [DSM 14042](#): Replace trimethylammonium chloride with 1.00 ml/l methanol solution (50% v/v) added from a sterile anoxic stock solution prepared under 100% N₂ gas. For incubation use 80% N₂ and 20% CO₂ gas mixture at two atmospheres of pressure.

For [DSM 16625](#): Supplement the autoclaved medium with 3.00 g/l Na₂S₂O₃ 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 × 7 H ₂ O	3.00	g
MnSO ₄ × H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ × 7 H ₂ O	0.10	g
CoSO ₄ × 7 H ₂ O	0.18	g
CaCl ₂ × 2 H ₂ O	0.10	g
ZnSO ₄ × 7 H ₂ O	0.18	g
CuSO ₄ × 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ × 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ × 2 H ₂ O	0.01	g
NiCl ₂ × 6 H ₂ O	0.03	g
Na ₂ SeO ₃ × 5 H ₂ O	0.30	mg
Na ₂ WO ₄ × 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



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p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml