141b: METHANOCULLEUS SP. MEDIUM

KCl 0.34 g
MgCl₂ x 6 H₂O 4.00 g
MgSO₄ x 7 H₂O 3.45 g
NH₄Cl 0.25 g
CaCl₂ x 2 H₂O 0.14 g
K₂HPO₄ 0.14 g
NaCl 6.00 g

Modified Wolin’s mineral solution 10.00 ml
Fe(NH₄)₂(SO₄)₂ x 6 H₂O (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
NaHCO₃ 5.00 g

Wolin’s vitamin solution 10.00 ml
L-Cysteine HCl x H₂O 0.50 g
Na₂S x 9 H₂O 0.50 g
Distilled water 1000.00 ml

1. Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H₂ 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% H₂ and 20% CO₂ gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. After sterilization add 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 final medium to 6.8 - 7.0.

2. For incubation use sterile 80% H₂ and 20% CO₂ gas mixture at two atmospheres of pressure.

3. Note: If the medium is being used without overpressure then adjust pH with a small amount of sterile anoxic 1 N HCl, if necessary.

For DSM 25996: Adjust pH of complete medium to 6.6.

Modified Wolin’s mineral solution (from medium 141)
Nitrilotriacetic acid 1.50 g
MgSO₄ x 7 H₂O 3.00 g
MnSO₄ x H₂O 0.50 g
NaCl 1.00 g
FeSO₄ x 7 H₂O 0.10 g
CoSO₄ x 7 H₂O 0.18 g

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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

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** (from medium 141)

- Biotin 2.00 mg
- Folic acid 2.00 mg
- Pyridoxine hydrochloride 10.00 mg
- Thiamine HCl 5.00 mg
- Riboflavin 5.00 mg
- Nicotinic acid 5.00 mg
- Calcium D-(-)-pantothenate 5.00 mg
- Vitamin B$_{12}$ 0.10 mg
- p-Aminobenzoic acid 5.00 mg
- (DL)-alpha-Lipoic acid 5.00 mg
Distilled water 1000.00 ml