141b: METHANOCULLEUS SP. MEDIUM

Final pH: 6.8 - 7.0
Final volume: 1013 ml

KCl	0.34	g
$MgCl_2$ x 6 H_2O	4.00	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
$CaCl_2$ x 2 H_2O	0.14	g
K_2HPO_4	0.14	g
NaCl	6.00	g
Modified Wolin's mineral solution	10.00	ml
$Fe(NH_4)_2(SO_4)_2$ x 6 H_2O (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
Wolin's vitamin solution (10x)	1.00	ml
$NaHCO_3$	5.00	g
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, vitamins, cysteine and sulfide), sparge medium with 80% H_2 and 20% CO_2 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% H_2 and 20% CO_2 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% N_2 gas atmosphere. Vitamins are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Adjust pH of final medium to 6.8 - 7.0.

2. For incubation use sterile 80% H_2 and 20% CO_2 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
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g

NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	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
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml