

## 1257: METHANOCELLA MEDIUM

NaCl	1.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.40	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
NH <sub>4</sub> Cl	0.10	g
KH <sub>2</sub> PO <sub>4</sub>	0.20	g
KCl	0.50	g
<b>Trace element solution</b>	1.00	ml
Yeast extract (OXOID)	0.10	g
Na-acetate	0.10	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins and reducing agents), then sparge medium with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic and adjust pH to 6.0. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials to 30% of their volume and autoclave. Prior to inoculation add vitamins, cysteine and sulfide from sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Vitamins are sterilized by filtration. If necessary, adjust the pH of the complete medium to 7.0.
2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

### Trace element solution (from medium 318)

Nitrolotriacetic acid (NTA)	12.80	g
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.00	g
MnCl <sub>2</sub> x 4 H <sub>2</sub> O	0.10	g
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnCl <sub>2</sub>	0.10	g
CuCl <sub>2</sub>	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.03	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
NaCl	1.00	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.03	g

Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	0.04	g
Distilled water	1000.00	ml

First dissolve NTA in 200 ml of distilled water and adjust pH to 6.5 with KOH, then dissolve mineral salts. Finally adjust pH to 6.5 with KOH and make up to 1000.00 ml.

### 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml