

**506: METHANOBACTERIUM ESPANOLAE MEDIUM**

Na-acetate x 3 H <sub>2</sub> O	2.50	g
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.45	g
K <sub>2</sub> HPO <sub>4</sub>	0.29	g
KH <sub>2</sub> PO <sub>4</sub>	0.18	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.12	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.06	g
NaCl	0.05	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	0.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.25	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.25	g
Distilled water	1000.00	ml

1. Dissolve ingredients except carbonate, vitamins, cysteine and sulfide, 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. Add and dissolve carbonate, then dispense medium into anoxic Hungate-type tubes or serum vials to 30% of their volume under 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere and autoclave. Add vitamins (sterilized by filtration), cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas. Prior to use check pH of complete medium and adjust to 5.8 - 6.0, if necessary.

2. Note: After growth has started and the culture is becoming turbid add sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 0.5 - 1 bar overpressure.

For DSM 19849: Adjust pH of complete medium to 7.0 - 7.2 with a sterile anoxic stock solution of Na<sub>2</sub>CO<sub>3</sub> prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. After inoculation tubes are pressurized with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to 1 bar overpressure.

**Modified Wolin's mineral solution** (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g

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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	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml