

**1011: SULFURIMONAS MJ MEDIUM**

NaCl	30.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.18	g
KCl	0.33	g
NH <sub>4</sub> Cl	0.25	g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O	0.01	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
NaHCO <sub>3</sub>	1.50	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> O	1.50	g
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Distilled water	1000.00	ml

1. Dissolve ingredients (except bicarbonate, thiosulfate and vitamins), then sparge medium with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials up to a volume of 20% and autoclave. Add bicarbonate, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions. Solutions of vitamins and thiosulfate are sterilized by filtration and stored under N<sub>2</sub>, whereas the solution of bicarbonate is prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and autoclaved. Adjust pH of complete medium to 6.7.

2. After inoculation pressurize vessels to 0.5 bar overpressure with sterile 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture and add sterile air in an amount that is equivalent to a volume of 20% of the headspace.

For DSM 23290: Supplement medium with 2.00 g/l NaNO<sub>3</sub>. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Do not add sterile air!

For DSM 24660: Omit thiosulfate and supplement medium with 4.00 g/l yeast extract and 4.00 g/l Trypton peptone. After autoclaving the medium is reduced with 0.30 g/l Na<sub>2</sub>S x 9 H<sub>2</sub>O added from a sterile anoxic stock solution (3% w/v) prepared under 100% N<sub>2</sub> gas and the pH adjusted to 6.5. After inoculation pressurize vessels to 1 bar overpressure with sterile 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Do not add sterile air!

For DSM 28671: Omit pressurizing vials with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

For DSM 101780, DSM 101688: Supplement medium with 2.00 g/l KNO<sub>3</sub>. Do not add overpressure of 80% N<sub>2</sub> and 20% CO<sub>2</sub> and do not add sterile air!

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

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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
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