

**283: PYRODICTION MEDIUM**

NaCl	13.85	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.50	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	2.75	g
KCl	0.33	g
NaBr	0.05	g
H <sub>3</sub> BO <sub>3</sub>	15.00	mg
SrCl <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	7.00	ml
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	10.00	mg
Citric acid (0.1% w/v)	5.00	ml
KI (0.01% w/v)	0.50	ml
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.75	g
KH <sub>2</sub> PO <sub>4</sub>	0.50	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O (0.1% w/v)	2.00	ml
<b>Modified Wolin's mineral solution</b>	10.00	ml
Sulfur (powdered)	30.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
Yeast extract (OXOID)	2.00	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Prepare the medium without sulfur, yeast extract and sodium sulfide, adjust pH to 5.0 - 5.5 with 10 N sulfuric acid, 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. Dispense medium under 80% H<sub>2</sub> and CO<sub>2</sub> gas atmosphere into anoxic Hungate-type tubes or serum vials that contain already the appropriate amount of sulfur, only to 30% of their volume to allow for a large headspace. For sterilization of medium heat vessels for at least 1 hour to 90 - 100°C on each of 3 successive days. Do not autoclave! Complete the medium by adding yeast extract and sulfide from sterile anoxic stock solutions prepared under 100% N<sub>2</sub> gas atmosphere. Adjust pH of complete medium to 5.5, if necessary.

2. After inoculation pressurize the vessels to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

For DSM 2709: Reduce amount of yeast extract to 0.20 g/l.

For DSM 6158, DSM 112164: Reduce amount of yeast extract to 0.50 g/l and omit citric acid.

**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

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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.