

## 730: FERROGLOBUS PLACIDUS MEDIUM

<b>Solution A</b>	950.00	ml
<b>Solution B</b>	30.00	ml
<b>Solution C</b>	1.00	ml
<b>Solution D</b>	10.00	ml
<b>Solution E</b>	10.00	ml

1. Sparge solution A with 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture for 30 - 45 min to make it anoxic, then dispense under same gas atmosphere into anoxic serum vials to 10% volume (e.g., 10 ml medium in 100 ml bottle) and autoclave. Solution B is autoclaved separately after sparging with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Solution C and D are prepared under 100% N<sub>2</sub> gas atmosphere and sterilized by filtration. Solution E is autoclaved under 100% N<sub>2</sub> gas atmosphere. To complete the medium add appropriate amounts of solutions B to E to the sterile solution A in the sequence as indicated.

2. Adjust pH of complete medium to 7.2, if necessary.

3. Note: Cultures are very sensitive to the nitrite produced during growth. Thus, incubate this strain only a few hours at one time (8-12 h) and thereafter check growth by microscopy. Immediately stop the incubation at 85°C, when the medium begins to show some turbidity.

### Solution A

NaCl	18.00	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
KCl	0.34	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
NH <sub>4</sub> Cl	0.24	g
KNO <sub>3</sub>	1.00	g
<b>Modified Wolin's mineral solution</b>	10.00	ml
Distilled water	940.00	ml

### Solution B

Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Distilled water	30.00	ml

### Solution C

<b>Wolin's vitamin solution (10x)</b>	1.00	ml
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### Solution D

Na-pyruvate	1.00	g
Distilled water	10.00	ml

### Solution E

Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
Distilled water	10.00	ml

### 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
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