

730. FERROGLOBUS PLACIDUS MEDIUM

Solution A:

NaCl	18.00	g
MgCl ₂ x 6 H ₂ O	4.30	g
CaCl ₂ x 2 H ₂ O	0.14	g
KCl	0.34	g
K ₂ HPO ₄	0.14	g
NH ₄ Cl	0.24	g
KNO ₃	1.00	g
Trace element solution (see medium 141)	10.00	ml
Distilled water	930.00	ml

Solution B:

Na ₂ CO ₃	1.50	g
Distilled water	30.00	ml

Solution C:

Vitamin solution (see medium 141)	10.00	ml
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Solution D:

Na-pyruvate	1.00	g
Distilled water	10.00	ml

Solution E:

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	10.00	ml

Sparge *solution A* with 80% H₂ and 20% CO₂ 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 under 1 bar overpressure of 80% H₂ and 20% CO₂ gas mixture. *Solution B* is autoclaved separately after sparging with 80% N₂ and 20% CO₂ gas mixture. *Solution C* and *D* are prepared under 100% N₂ gas atmosphere and sterilized by filtration. *Solution E* is autoclaved under 100% N₂ gas atmosphere. To complete the medium add appropriate amounts of *solutions B to E* to the sterile *solution A* in the sequence as indicated. Adjust pH of complete medium to 7.0 – 7.2, if necessary. After inoculation pressurize the bottles with 80% H₂ and 20% CO₂ gas mixture to 2 bar overpressure.

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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.