792: PYROLOBUS FUMARII MEDIUM

Solution A 989.00 ml  
Solution B 10.00 ml  
Solution C 1.00 ml  
Solution D 10.00 ml

1. 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 (e.g., 20 ml medium in 100 ml serum bottles) and autoclave. Solutions B and D are autoclaved separately under 100% N₂ gas atmosphere and solution C is prepared under 100% N₂ gas and sterilized by filtration. To complete the medium appropriate amounts of solutions B, C and D are added to solution A. Adjust pH of the complete medium to 5.8 using a sterile anoxic stock solution of 5% (w/v) Na₂CO₃, if necessary.

2. After inoculation pressurize vials to 1 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

3. Note: Solution A has to be prepared freshly. It cannot be stored for a long period of time.

Solution A  
NaCl 13.85 g  
MgSO₄ x 7 H₂O 3.50 g  
MgCl₂ x 6 H₂O 2.75 g  
CaCl₂ x 2 H₂O 0.38 g  
KCl 0.33 g  
NaBr 0.05 g  
H₃BO₃ 15.00 mg  
SrCl₂ x 6 H₂O (0.1% w/v) 7.00 ml  
KI (0.01% w/v) 0.50 ml  
Wolfe’s mineral elixir 1.00 ml  
Sodium resazurin (0.1% w/v) 0.50 ml  
Double distilled water 980.00 ml

Solution B  
KNO₃ 1.00 g  
KH₂PO₄ 0.50 g  
Distilled water 10.00 ml

Solution C  
Seven vitamins solution 1.00 ml
Solution D
Na$_2$S x 9 H$_2$O 0.15 g
Distilled water 10.00 ml

Wolfe's mineral elixir (from medium 792)
MgSO$_4$ x 7 H$_2$O 30.00 g
MnSO$_4$ x H$_2$O 5.00 g
NaCl 10.00 g
FeSO$_4$ x 7 H$_2$O 1.00 g
CoCl$_2$ x 6 H$_2$O 1.80 g
CaCl$_2$ x 2 H$_2$O 1.00 g
ZnSO$_4$ x 7 H$_2$O 1.80 g
CuSO$_4$ x 5 H$_2$O 0.10 g
AlK(SO$_4$)$_2$ x 12 H$_2$O 0.18 g
H$_3$BO$_3$ 0.10 g
Na$_2$MoO$_4$ x 2 H$_2$O 0.10 g
(NH$_4$)$_2$Ni(SO$_4$)$_2$ x 6 H$_2$O 2.80 g
Na$_2$WO$_4$ x 2 H$_2$O 0.10 g
Na$_2$SeO$_4$ 0.10 g
Distilled water 1000.00 ml

First adjust pH to 1.0 with diluted H$_2$SO$_4$, then add and dissolve the salts.

Seven vitamins solution (from medium 503)
Vitamin B$_{12}$ 100.00 mg
p-Aminobenzoic acid 80.00 mg
D-(-)-biotin 20.00 mg
Nicotinic acid 200.00 mg
Calcium pantothenate 100.00 mg
Pyridoxine hydrochloride 300.00 mg
Thiamine-HCl x 2 H$_2$O 200.00 mg
Distilled water 1000.00 ml