

283: PYRODICTIONIUM MEDIUM

Final pH: 5.5

Final volume: 1025 ml

| | | |
|---|---------|----|
| NaCl | 13.85 | g |
| MgSO ₄ x 7 H ₂ O | 3.50 | g |
| MgCl ₂ x 6 H ₂ O | 2.75 | g |
| KCl | 0.33 | g |
| NaBr | 0.05 | g |
| H ₃ BO ₃ | 15.00 | mg |
| SrCl ₂ x 6 H ₂ O (0.1% w/v) | 7.00 | ml |
| (NH ₄) ₂ SO ₄ | 10.00 | mg |
| Citric acid (0.1% w/v) | 5.00 | ml |
| KI (0.01% w/v) | 0.50 | ml |
| CaCl ₂ x 2 H ₂ O | 0.75 | g |
| KH ₂ PO ₄ | 0.50 | g |
| NiCl ₂ x 6 H ₂ O (0.1% w/v) | 2.00 | ml |
| Modified Wolin's mineral solution | 10.00 | ml |
| Sulfur (powdered) | 30.00 | g |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| Yeast extract (OXOID) | 2.00 | g |
| Na ₂ S x 9 H ₂ 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% H₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under 80% H₂ and CO₂ 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% N₂ 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% H₂ and 20% CO₂ 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.

For DSM 113394: Adjust the amount of yeast extract to 0.5 g/l.

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Modified Wolin's mineral solution (from medium 141)

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|--|---------|----|
| Nitrilotriacetic acid | 1.50 | g |
| MgSO ₄ x 7 H ₂ O | 3.00 | g |
| MnSO ₄ x H ₂ O | 0.50 | g |
| NaCl | 1.00 | g |
| FeSO ₄ x 7 H ₂ O | 0.10 | g |
| CoSO ₄ x 7 H ₂ O | 0.18 | g |
| CaCl ₂ x 2 H ₂ O | 0.10 | g |
| ZnSO ₄ x 7 H ₂ O | 0.18 | g |
| CuSO ₄ x 5 H ₂ O | 0.01 | g |
| AlK(SO ₄) ₂ x 12 H ₂ O | 0.02 | g |
| H ₃ BO ₃ | 0.01 | g |
| Na ₂ MoO ₄ x 2 H ₂ O | 0.01 | g |
| NiCl ₂ x 6 H ₂ O | 0.03 | g |
| Na ₂ SeO ₃ x 5 H ₂ O | 0.30 | mg |
| Na ₂ WO ₄ x 2 H ₂ 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.