

ATCC Medium: 2039 *Acidithiobacillus ferrooxidans* Medium

Solution A

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|---|----------|
| (NH ₄) ₂ SO ₄ | 0.8 g |
| MgSO ₄ x 7H ₂ O..... | 2.0 g |
| K ₂ HPO ₄ | 0.4 g |
| *Wolfe's Mineral Solution (see below)..... | 5.0 ml |
| DI Water..... | 800.0 ml |

Adjust Solution A to pH 2.3 with H₂SO₄ and filter-sterilize.

*ATCC's sterile ready-to-use Trace Mineral Supplement (catalog no. MD-TMS) can be substituted.

Solution B

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| FeSO ₄ x 7H ₂ O..... | 20.0 g |
| DI Water..... | 200.0 ml |

Stir Solution B to dissolve and quickly filter-sterilize.

Complete Medium

Aseptically combine Solutions A and B. (A yellow precipitate is normal; it becomes darker as the iron oxidizes). Dispense as required.

Wolfe's Mineral Solution

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|--|---------|
| Nitrilotriacetic acid..... | 1.5 g |
| MgSO ₄ x 7H ₂ O..... | 3.0 g |
| MnSO ₄ x H ₂ O..... | 0.5 g |
| NaCl..... | 1.0 g |
| FeSO ₄ x 7H ₂ O..... | 0.1 g |
| CoCl ₂ x 6H ₂ O..... | 0.1 g |
| CaCl ₂ | 0.1 g |
| ZnSO ₄ x 7H ₂ O..... | 0.1 g |
| CuSO ₄ x 5H ₂ O..... | 0.01 g |
| AlK(SO ₄) ₂ x 12H ₂ O..... | 0.01 g |
| H ₃ BO ₃ | 0.01 g |
| Na ₂ MoO ₄ x 2H ₂ O..... | 0.01 g |
| DI Water..... | 1000 ml |

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1000 ml with remaining water and add remaining compounds one at a time.