

ATCC medium: 1374 Cholesterol medium

Solution A:

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|---|----------|
| KH ₂ PO ₄ | 1.0 g |
| MgSO ₄ . 7H ₂ O | 0.5 g |
| NaCl..... | 0.1 g |
| CaCl ₂ . 2H ₂ O | 0.1 g |
| (NH ₄) ₂ SO ₄ | 5.0 g |
| Wolfe's Mineral Solution (see below)..... | 10.0 ml |
| Distilled water..... | 500.0 ml |

Solution B:

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|--|----------|
| Yeast extract..... | 0.5 g |
| Tween 80..... | 1.0 g |
| Cholesterol..... | 2.0 g |
| Agar, Noble (BD 214230) (if needed)..... | 15.0 g |
| Distilled water..... | 500.0 ml |

Autoclave Solutions A and B separately at 121C for 15 minutes. Cool; mix solutions and aseptically add:

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|--------------------------------------|---------|
| Amino Acid Solution (see below)..... | 20.0 ml |
| Vitamin Solution (see below)..... | 10.0 ml |

Adjust medium to a final pH of 6.8.

Amino Acid Solution:

| | |
|----------------------|----------|
| L-Histidine..... | 0.5 g |
| DL-Methionine..... | 0.1 g |
| DL-Tryptophan..... | 0.1 g |
| Distilled water..... | 100.0 ml |

Filter-sterilize.

Vitamin Solution:

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|------------------------------|-----------|
| Biotin..... | 200.0 mcg |
| Calcium D-(+)-pantothenate.. | 40.0 mg |
| Folic acid..... | 200.0 mcg |
| i-Inositol..... | 200.0 mg |
| Niacin..... | 40.0 mg |
| p-Aminobenzoic acid..... | 20.0 mg |
| Pyridoxine hydrochloride.... | 40.0 mg |
| Riboflavin..... | 20.0 mg |
| Thiamine..... | 40.0 mg |
| Distilled water..... | 1.0 L |

Filter-sterilize.

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

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

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.