

ATCC medium: 2044 Acetone medium

ATCC Medium 1778 (see below) with 5 g/L sodium pyruvate, 0.58 g/L acetone, and 3.4 g/L KNO₃ (each separately sterilized)

ATCC Medium 1778:

Solution A:

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|---|----------|
| Na ₂ SO ₄ | 3.0 g |
| KH ₂ PO ₄ | 0.2 g |
| NH ₄ Cl | 0.3 g |
| NaCl..... | 7.0 g |
| MgCl ₂ . 6H ₂ O | 1.3 g |
| KCl..... | 0.5 g |
| CaCl ₂ . 2H ₂ O | 0.15 g |
| Resazurin..... | 1.0 mg |
| Distilled water..... | 870.0 ml |

Solution B:

Trace Elements Solution SL-10 (see below).....1.0 ml.

Solution C:

| | |
|--------------------------|----------|
| NaHCO ₃ | 5.0 g |
| Distilled water..... | 100.0 ml |

Solution D:

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|--|---------|
| Sodium acetate . 3H ₂ O | 2.5 g |
| Distilled water..... | 10.0 ml |

Solution E:

Wolfe's Vitamin Solution (see below)10.0 ml

Solution F:

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| Na ₂ S . 9H ₂ O | 0.4 g |
| Distilled water..... | 10.0 ml |

Directions for complete medium:

Boil Solution A for 3 minutes, cool to room temperature under 80% N₂, 20% CO₂ to reach a pH below 6. Autoclave anaerobically under the same gas phase. Autoclave Solutions B, D, E and F separately under nitrogen. Filter-sterilize Solution C and flush with 80% N₂, 20% CO₂ to remove oxygen. Add Solutions B through F to sterile, cooled Solution A in the sequence indicated. Tube complete medium anaerobically under 80% N₂, 20% CO₂. Final pH 7.1 - 7.4.

Trace Elements Solution SL-10:

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|---|----------|
| HCl (25%) | 10.0 ml |
| FeCl ₂ . 4H ₂ O | 1.5 g |
| ZnCl ₂ | 70.0 mg |
| MnCl ₂ . 4H ₂ O | 100.0 mg |
| H ₃ BO ₃ | 6.0 mg |
| CoCl ₂ . 6H ₂ O | 190.0 mg |
| CuCl ₂ . 2H ₂ O | 2.0 mg |
| NiCl ₂ . 6H ₂ O | 24.0 mg |
| Na ₂ MoO ₄ . 2H ₂ O..... | 36.0 mg |
| Distilled water..... | 990.0 ml |

Dissolve FeCl₂ in the HCl, dilute with water, add and dissolve the other salts; adjust pH to 6.0 with NaOH, and fill to 1.0 L with distilled water.

Wolfe's Vitamins:

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|---|---------|
| Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS). | |
| Folic acid..... | 2.0 mg |
| Pyridoxine hydrochloride.... | 10.0 mg |
| Riboflavin..... | 5.0 mg |
| Biotin..... | 2.0 mg |
| Thiamine..... | 5.0 mg |
| Nicotinic acid..... | 5.0 mg |
| Pantothenic acid..... | 5.0 mg |
| Vitamin B12..... | 0.1 mg |
| p-Aminobenzoic acid..... | 5.0 mg |
| Thioctic acid..... | 5.0 mg |
| Distilled water..... | 1.0 L |