

ATCC medium: 1770 *Clostridium halophilum* medium

Solution 1:

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|---|----------|
| NaCl..... | 60.0 g |
| MgSO ₄ . 7H ₂ O | 5.0 g |
| NH ₄ Cl | 1.0 g |
| CaCl ₂ . 2H ₂ O | 25.0 mg |
| Trace Elements Solution SL-10 (see below)..... | 1.0 ml |
| Wolfe's Vitamin Solution (see below)..... | 10.0 ml |
| Na ₂ SeO ₃ . 5H ₂ O..... | 15.0 mcg |
| Resazurin..... | 1.0 mg |
| Betaine..... | 5.86 g |
| Yeast extract..... | 1.0 g |
| L-Alanine..... | 2.2 g |
| Distilled water..... | 900.0 ml |

Solution 2:

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|--------------------------|---------|
| NaHCO ₃ | 5.0 g |
| Distilled water..... | 80.0 ml |

Bubble under N₂ for 20 minutes; add to Solution 1.

Solution 3:

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|---------------------------------------|----------|
| K ₂ HPO ₄ | 358.0 mg |
| KH ₂ PO ₄ | 223.0 mg |
| Distilled water..... | 10.0 ml |

Autoclave under N₂.

Solution 4:

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|---|----------|
| Na ₂ S . 9H ₂ O | 300.0 mg |
| Distilled water..... | 10.0 ml |

Autoclave under N₂.

Boil and cool Solution 1 under 80% N₂, 20% CO₂. Add Solution 2. Adjust to pH 8.3 +/- 0.1, tube and autoclave under 80% N₂, 20% CO₂ gas phase. Add Solutions 3 and 4 aseptically to cooled, sterile medium.

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 Vitamin Solution:

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

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|----------------------------|---------|
| Biotin | 2.0 mg |
| Folic acid | 2.0 mg |
| Pyridoxine hydrochloride | 10.0 mg |
| Thiamine . HCl | 5.0 mg |
| Riboflavin | 5.0 mg |
| Nicotinic acid | 5.0 mg |
| Calcium D-(+)-pantothenate | 5.0 mg |
| Vitamin B12 | 0.1 mg |
| p-Aminobenzoic acid | 5.0 mg |
| Thioctic acid | 5.0 mg |
| Distilled water | 1.0 L |