

ATCC medium: 1403 *Methylophaga* medium

Complete Medium:

Aseptically combine the following solutions just before use:

Mineral Base (see below).....100.0 ml
Solution T (see below).....2.0 ml
Vitamin B12 (0.1 mcg/ml) filter-sterilized.....1.0 ml
Methanol, filter-sterilized.....0.3 ml

For solid medium prepare a 2X Mineral Base solution and a 3% agar solution and autoclave both separately. Cool to 50C and aseptically mix the solutions in equal volume before adding solution T, the B12 solution and methanol.

Mineral Base:

NaCl.....24.0 g
MgCl₂ . 6H₂O3.0 g
MgSO₄ . 7H₂O2.0 g
KCl.....0.5 g
CaCl₂ . 2H₂O1.0 g
Bis-Tris.....0.5 g
Wolfe's Mineral Solution (see below).....10.0 ml
Distilled water.....990.0 ml

Adjust to pH 7.3 with HCl. Autoclave at 121C for 15 minutes.

Solution T:

KH₂PO₄0.7 g
NH₄Cl10.0 g
Bis-Tris.....10.0 g
Ferric ammonium citrate.....0.3 g
Distilled water.....100.0 ml

Adjust to pH 7.3. Autoclave at 121C for 15 minutes.

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.