

ATCC medium: 2042 Alkaliphilic Spirochaete medium

Yeast extract.....	0.5 g
Sucrose.....	5.0 g
NaCl.....	48.0 g
NH ₄ Cl	0.5 g
KH ₂ PO ₄	0.3 g
Na ₂ CO ₃	2.0 g
NaHCO ₃	4.5 g
Wolfe's Mineral Solution (see below)	10.0 ml
Resazurin.....	1.0 mg
Na ₂ S . 9H ₂ O	0.5 g
Distilled water.....	1.0 L

Adjust medium, if necessary, for final pH 9.1 +/- 0.1. Boil, cool and dispense anaerobically under 100% N₂. 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.)

Nitritilotriacetic 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 nitritilotriacetic 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.