

ATCC medium: 1915 *Pyrococcus* medium (DSM medium 377)

KH₂PO₄0.5 g
NiCl₂ . 6H₂O2.0 mg
Wolfe's Mineral Solution (see below)....10.0 ml
Yeast Extract (BD 212750).....1.0 g
Peptone, Bacto (BD 211677).....5.0 g
Resazurin.....1.0 mg
Sulfur, powdered.....30.0 g
Na₂S . 9H₂O0.5 g
Seawater.....1.0 L

Add approximately 0.18 g powdered sulfur per test tube. Dissolve remaining ingredients, except sodium sulfide, in seawater. Adjust solution to pH 6.4 +/- 0.1. Under 100% N₂ gas, boil, cool and dispense 6.0 ml aliquots anaerobically into tubes containing the powdered sulfur. Autoclave medium at 100C for 30 minutes on 3 consecutive days. Prior to inoculation, reduce the medium with a sterile neutral solution of sodium sulfide to 0.5 g/L final concentration. Final pH of complete medium is 6.5.

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
MgSO₄ . 7H₂O3.0 g
MnSO₄ . H₂O0.5 g
NaCl.....1.0 g
FeSO₄ . 7H₂O0.1 g
CoCl₂ . 6H₂O0.1 g
CaCl₂.....0.1 g
ZnSO₄ . 7H₂O0.1 g
CuSO₄ . 5H₂O0.01 g
AlK(SO₄)₂ . 12H₂O.....0.01 g
H₃BO₃0.01 g
Na₂MoO₄ . 2H₂O.....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.