

ATCC medium: 2059 Modified MRCM medium

Peptone, Bacto (BD 211677).....10.0 g
Yeast extract.....10.0 g
Beef extract.....5.0 g
Soluble starch.....1.0 g
Resazurin.....1.0 mg
NaCl.....0.3 g
Solution A (see below).....10.0 ml
Solution B (see below).....10.0 ml
Solution C (see below).....50.0 ml
Solution D (see below).....10.0 ml
Distilled water.....920.0 ml

Mix ingredients through NaCl in 920 ml distilled water and autoclave at 121C for 15 minutes. Cool under 80% N₂, 20% CO₂. Aseptically add solutions A through D using anaerobic techniques. Dispense completed medium as desired using anaerobic techniques and the same gas phase. Final pH 7.2.

Solution A:

Modified Wolfe's Minerals (see below)...10.0 ml

Filter-sterilize and equilibrate under 100% N₂.

Solution B:

Sodium pyruvate.....1.1 g
Distilled water.....10.0 ml

Filter-sterilize and equilibrate under 100% N₂.

Solution C:

NaHCO₃.....3.2 g
Distilled water.....50.0 ml

Filter-sterilize and equilibrate under 100% N₂.

Solution D:

Na₂S . 9H₂O0.36 g
Distilled water.....10.0 ml

Equilibrate under 100% N₂ and autoclave at 121C for 15 minutes.

Modified Wolfe's Minerals:

Na₂SeO₃10.0 mg
NiCl₂ . 6H₂O10.0 mg
Na₂WO₄ . 2H₂O.....10.0 mg
Wolfe's Mineral Solution (see below).....1.0 L

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.