

ATCC medium: 2189 *Syntrophococcus* medium

Basal Medium:

Mineral Solution (See below).....50.0 ml
SL-10 Trace Elements Solution (See below).....1.0 ml
Rumen fluid, clarified.....300.0 ml
Trypticase or Casitone.....5.0 g
Sodium formate.....0.6 g
Wolfe's Vitamin Solution (See below).....10.0 ml
Resazurin.....1.0 mg
Distilled water.....560.0 ml

Bring distilled water to boiling. Add ingredients. Cool under 80% N₂, 20% CO₂.

Adjust pH to 6.4. Autoclave at 121C for 15 minutes.

Aseptically add all of Solutions 1 through 3 and 1.0 ml of Solution 4.

Readjust pH to 6.4-6.8. Dispense under gas.

Mineral Solution:

KH₂PO₄10.0 g
MgCl₂ . 6H₂O6.6 g
NaCl.....8.0 g
NH₄Cl8.0 g
CaCl₂ . 2H₂O1.0 g
Distilled water.....1.0 L

SL-10 Trace Elements Solution:

HCl (25%).....10.0 ml
FeCl₂ . 4H₂O1.5 g
ZnCl₂70.0 mg
MnCl₂ . 4H₂O100.0 mg
H₃BO₃6.0 mg
CoCl₂ . 6H₂O190.0 mg
CuCl₂ . 2H₂O2.0 mg
NiCl₂ . 6H₂O24.0 mg
Na₂MoO₄ . 2H₂O.....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).

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

Filter-sterilize.

Solution 2:

NaHCO₃.....2.5 g
Distilled water.....50.0 ml

Filter-sterilize.

Solution 3:

L-Cysteine . HCl.....0.24 g
Distilled water.....10.0 ml

Filter-sterilize.

Solution 4:

Na₂S . 9H₂O0.78 g
Distilled water.....10.0 ml

Filter-sterilize. Add 1.0 ml to base medium; discard unused portion.