

ATCC medium: 1644 *Clostridium pfennigii* medium

Solution A:

Mineral Solution (See below).....	50.0 ml
SL-10 Trace Elements Solution (See below).....	1.0 ml
Yeast extract.....	2.0 g
Sodium vanillate.....	2.0 g
Ferulic acid.....	0.58 g
Wolfe's Vitamin Solution (See below).....	5.0 ml
Resazurin.....	1.0 mg
Rumen fluid.....	300.0 ml
Distilled water.....	540.0 ml

Adjust for final pH of 7.0-7.2. Prepare Solution A using anaerobic techniques under 80% N₂, 20% CO₂. Sterilize by autoclaving.

Mineral Solution:

KH ₂ PO ₄	10.0 g
MgCl ₂ . 6H ₂ O	6.6 g
NaCl.....	8.0 g
NH ₄ Cl	8.0 g
CaCl ₂ . 2H ₂ O	1.0 g
Distilled water.....	1.0 L

SL-10 Trace Elements Solution:

HCl (25%).....	10.0 ml
FeCl ₂ . 4H ₂ O	1.5 g
ZnCl ₂	70.0 mg
MnCl ₂ . 4H ₂ O	100.0 mg
H ₃ BO ₃	6.0 mg
CoCl ₂ . 6H ₂ O	190.0 mg
CuCl ₂ . 2H ₂ O	2.0 mg
NiCl ₂ . 6H ₂ O	24.0 mg
Na ₂ MoO ₄ . 2H ₂ O.....	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 and outgas for 20 minutes with the N₂, CO₂ gas mixture.

Solution C:

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

Autoclave under N₂ gas.

Solution D:

Na₂S . 9H₂O78.0 mg
Distilled water.....1.0 ml

Autoclave under N₂ gas.

Aseptically combine Solutions A through D under 80% N₂, 20% CO₂. Final pH of complete medium should be 7.0-7.2.