

ATCC medium: 2273 *Anoxybacillus* medium

KH ₂ PO ₄	0.20 g
MgCl ₂ . 6H ₂ O	0.10 g ₂
KCl.....	0.20 g
NH ₄ Cl	1.0 g
Na ₂ CO ₃	2.76 g
NaHCO ₃	10.0 g
NaCl.....	5.0 g
Na ₂ S . 9H ₂ O	0.5 g
Yeast extract.....	0.5 g
Glucose.....	5.0 g
Wolfe's Vitamin Solution (see below).....	10.0 ml
SL-10 Trace Elements Solution (see below).....	1.0 ml
Resazurin.....	0.5 mg
Distilled water.....	1.0 L

Prepare the medium without sulfide, vitamins, or glucose and flush with N₂ gas for 30-60 minutes. Add sulfide, adjust the pH to 9.5-9.7 with NaOH, dispense in anaerobic vessels and autoclave. Add vitamins and glucose from sterile, anaerobic stock solutions prepared under N₂.

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

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.