

ATCC medium: 2683 Natranaerobius

KH ₂ PO ₄	0.20 g
MgCl ₂	0.10 g
NH ₄ Cl	0.50 g
NaCl.....	100.00 g
Yeast extract (Difco).....	10.00 g
Tryptone(Difco).....	10.00 g
Wolfe's Mineral Solution (see below).....	1.00 ml
Wolfe's Vitamin Solution (see below)....	10.00 ml
Na ₂ CO ₃	68.00 g
NaHCO ₃	38.00 g
Cysteine HCl.....	0.70 g
Casamino Acids.....	5.00 g
Distilled water.....	1000.00 ml

Mix all the ingredients except Na₂CO₃, NaHCO₃ and cysteine HCl under a gas phase of 100% N₂. Boil and cool to room temperature. pH the solution to 8.5 (measured at 60C) and autoclave at 121°C for 15 minutes. Filter-sterilize Na₂CO₃, NaHCO₃ and cysteine HCl and flush with 100% N₂ to remove oxygen. After autoclaving allow the base medium to cool and add Na₂CO₃, NaHCO₃ and cysteine HCl. Just before inoculation add the following stock solution anaerobically: 0.1 ml of a 2M glucose solution per 10.0 ml of media.

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....	1.500 g
MgSO ₄ . 7H ₂ O	3.000 g
MnSO ₄ . H ₂ O	0.500 g
NaCl.....	1.000 g
FeSO ₄ . 7H ₂ O	0.100 g
Co(NO ₃) ₂ . 6H ₂ O.....	0.100 g
CaCl ₂	0.100 g
ZnSO ₄ . 7H ₂ O	0.100 g
CuSO ₄ . 5H ₂ O	0.010 g
AlK(SO ₄) ₂ . 12H ₂ O.....	0.010 g
H ₃ BO ₃	0.010 g
Na ₂ MoO ₄ . 2H ₂ O.....	0.010 g
Na ₂ SeO ₃ (anhydrous)	0.001 g
Na ₂ WO ₄ . 2H ₂ O.....	0.010 g
NiCl ₂ . 6H ₂ O	0.020 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.

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