

ATCC medium: 2642 Prolixibacter bellarivorans Medium (A1 media)

Solution A:

NaCl.....20.00 g
KCl.....0.77 g
NH₄Cl.....0.25 g
KH₂PO₄.....0.10 g
MgSO₄ . 7H₂O.....0.20 g
Wolfe's Mineral Solution (see below)....10.00 ml
Wolfe's Vitamin Solution (see below)....10.00 ml
Distilled water.....870.00 ml

Solution B:

NaHCO₃.....5.0 g
Distilled water.....100.0 ml

Mix all the ingredients in solution A under a gas phase of 100% N₂. pH the solution to 6.8 and autoclave at 121°C for 15 minutes. Filter-sterilize solution B and flush with 100% N₂ to remove oxygen. Cool solution A and add Solution B. Just before inoculation add the following stock solutions anaerobically: 0.2 ml of APM salts, 0.1 ml Sodium Sulfide and 0.1 ml Galactose per 9.0 ml of media.

APM Salts (per 100ml):

MgCl₂ . 6H₂O.....21.20 g
CaI₂ . 2H₂O.....3.04 g
Autoclave at 121°C for 15 minutes

Sulfide Stock (per 10ml):

Na₂S . 9H₂O.....0.40 g
Autoclave at 121°C for 15 minutes

Galactose stock (0.5M) (per 10ml):

Galactose.....0.90 g
Filter sterilize with a 0.2 µm filter.

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
MgSO₄ . 7H₂O.....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