

ATCC medium: 1962 *Isosphaera* medium

Solution A:

NaCl.....	250.0 mg
(NH ₄) ₂ SO ₄	125.0 mg
KCl.....	125.0 mg
MgSO ₄ . 7H ₂ O	100.0 mg
CaCl ₂ . 2H ₂ O	80.0 mg
KH ₂ PO ₄	75.0 mg
FeCl ₃	73.0 mcg
Trace Element Solution SL-7 (see below).....	2.5 ml
Agar, Noble (BD 214050), if needed.....	15.0 g
Distilled water.....	800.0 ml

For solid medium, dissolve agar in 400 ml distilled water and mix salts in another 400 ml water. Autoclave both the solutions separately at 121C for 15 minutes and combine aseptically to retard formation of agar-salt complexes.

For agar and liquid media, adjust Solution A to pH 7.6 with NaOH and remove any precipitate that forms by filtering through Whatman #1 paper. Autoclave Solution A at 121C for 15 minutes.

Aseptically add the following solutions to sterile Solution A:

Solution B (see below).....	100.0 ml
Solution C (see below).....	100.0 ml

Solution B:

NaHCO ₃	0.42 g
Distilled water.....	100.0 ml

Filter-sterilize Solution B.

Solution C:

Glucose.....	0.25 g
Casamino acids.....	0.25 g
Distilled water.....	100.0 ml

Filter-sterilize Solution C.

Vitamin Solution:

Nicotinic acid.....200.0 mg
Thiamine . HCl.....200.0 mg
p-Aminobenzoic acid.....20.0 mg
Biotin.....2.0 mg
Vitamin B12.....25.0 mcg
Distilled water.....100.0 ml

Filter-sterilize.

Trace Element Solution SL-7:

Hydrochloric acid, 25%.....10.0 ml
FeCl₂ . 4H₂O1.5 g
CoCl₂ . 6H₂O190.0 mg
MnCl₂ . 4H₂O100.0 mg
ZnCl₂70.0 mg
H₃BO₃62.0 mg
Na₂MoO₄ . 2H₂O.....36.0 mg
NiCl₂ . 6H₂O24.0 mg
CuCl₂ . 2H₂O17.0 mg
Distilled water.....1.0 L

Dissolve the FeCl₂ . 4H₂O in the concentrated HCl, then dilute.