

**ATCC medium: 1354 Nitrate mineral salts medium (ATCC medium 1306) with
0.1% sterile methanol**

MgSO ₄ . 7H ₂ O	1.0 g
CaCl ₂ . 6H ₂ O	0.20 g
Chelated Iron Solution (see below)	2.0 ml
KNO ₃	1.0 g
Trace Element Solution (see below)	0.5 ml
KH ₂ PO ₄	0.272 g
Na ₂ HPO ₄ . 12H ₂ O.....	0.717 g
Purified Agar (e.g., Oxoid L28).....	12.5 g
Methanol.....	1.0 ml
Distilled water.....	1.0 L

combine all ingredients but methanol. Adjust pH to 6.8. Autoclave at 121C for 15 minutes. After autoclaving, add filter-sterilized methanol.

Chelated Iron Solution:

Ferric (III) ammonium citrate*.....	0.1 g
EDTA, sodium salt.....	0.2 g
HCl (concentrated)	0.3 ml
Distilled deionized water.....	100.0 ml

*0.5 g of Ferric (III) chloride may be substituted.

Use 2.0 ml of this chelated iron solution per liter of final medium.

Trace Element Solution:

EDTA.....	500.0 mg
FeSO ₄ . 7H ₂ O	200.0 mg
ZnSO ₄ . 7H ₂ O	10.0 mg
MnCl ₂ . 4H ₂ O	3.0 mg
H ₃ BO ₃	30.0 mg
CoCl ₂ . 6H ₂ O	20.0 mg
CaCl ₂ . 2H ₂ O	1.0 mg
NiCl ₂ . 6H ₂ O	2.0 mg
Na ₂ MoO ₄ . 2H ₂ O.....	3.0 mg
Distilled water.....	1.0 L

Autoclave at 121C for 15 minutes.

