

ATCC medium: 1661 M13 *Verrucomicrobium* medium

Peptone.....0.25 g
Yeast extract.....0.25 g
Glucose.....0.25 g
Vitamin Solution (see below).....10.0 ml
Modified Hutner's Basal Salts (see below).....20.0 ml
0.1 M Tris/HCl at pH 7.5.....50.0 ml
Artificial Seawater (see below).....250.0 ml
Distilled water.....670.0 ml

Autoclave at 121C for 15 minutes.

Vitamin Solution from Medium 289 (see below) plus 5.0 mg/L nicotinamide.

Vitamin Solution:

Biotin.....2.0 mg
Folic acid.....2.0 mg
Thiamine . HCl.....5.0 mg
Calcium D-(+)-pantothenate...5.0 mg
Vitamin B12.....0.1 mg
Riboflavin.....5.0 mg
Distilled water.....1.0 L

Artificial Seawater:

NaCl.....23.477 g
MgCl₂.....4.981 g
Na₂SO₄.....3.917 g
CaCl₂.....1.102 g
KCl.....0.664 g
NaHCO₃.....0.192 g
KBr.....0.006 g
H₃BO₃.....0.026 g
SrCl₂.....0.024 g
NaF.....0.003 g
Distilled water to.....1.0 L

Modified Hutner's Basal Salts:

Nitrilotriacetic acid.....10.0 g
MgSO₄ . 7H₂O.....29.7 g
CaCl₂ . 2H₂O.....3.34 g
Ammonium molybdate.....9.25 mg
FeSO₄ . 7H₂O.....99.0 mg
Metals "44" (see below).....50.0 ml
Distilled water to.....1.0 L

Dissolve and neutralize the nitrilotriacetic acid with KOH (7.3 g); add the other ingredients and adjust the pH to 6.6 - 6.8 before bringing the volume to 1.0 L with distilled water.

Metals "44":

EDTA.....0.25 g
ZnSO₄ . 7H₂O1.1 g
FeSO₄ . 7H₂O0.5 g
MnSO₄ . 7H₂O0.154 g
CuSO₄ . 5H₂O0.04 g
Co(NO₃)₂ . 6H₂O.....0.025 g
Na₂B₄O₇ . 10H₂O.....0.018 g
Distilled water.....100.0 ml

Initially add a few drops of H₂SO₄ to the distilled water to retard precipitation.