

ATCC Medium: 2626 Peat Medium 1

Major Metals (see below).....	10 ml
Trace Metal Solution (see below).....	1 ml
DI Water.....	1000 ml

Adjust to pH 5 with approx 1-2 drops of 0.1-0.2N HCl.

Major Metals

NaCl.....	1.36 g
KCl.....	2.68 g
DI Water.....	1000 ml

Trace Metal solution

CoCl ₂ x 6 H ₂ O.....	0.024 g
ZnCl ₂	0.075 g
H ₃ BO ₃	0.019 g
NiCl ₂ x 6H ₂ O.....	0.024 g
NaMo ₄ x 2 H ₂ O.....	0.024 g
FeCl ₂ 4 H ₂ O.....	1.344 g
MnSO ₄ x 4 H ₂ O.....	0.026 g
MgSO ₄ 7 H ₂ O.....	1.556 g
CaCl ₂ x 2H ₂ O.....	2.336 g
CuSO ₄ x 5 H ₂ O.....	0.009 g
AlK(SO ₄) ₂ X 12H ₂ O.....	3.446 g

Put tubes in anaerobic chamber (needs N₂) at least 1 hour before or overnight.
Dispense 5ml of media into Balsh tubes and crimp. Autoclave at 121 C.
For best results media should be used within 3-4 weeks

Add the following solutions at time of inoculation.

Add the following to each tube/flask	In 5ml tubes	Final Concentration
83 mM TiNta	0.06ml	1mM
0.5 M Homopipes	0.05ml	5mM
Vitamin Solution (NOT High B-12)	0.05ml	0.2,1.0,0.5,or0.01mg/L of each
2% YE	0.05 ml	0.2mg
50 mM CoM	0.05ml	0.5 mM
20 mM Acetate	0.05ml	0.2 mM

Solution Preparation:

TiNta

7.2 mL of 0.38M NaOH

4.8 mL of 0.5M NaN₃

0.55mL of TiCl₃ (15% solution in HCl)

Filter sterilize and gas with N₂

0.5M Homopipes (~pH 5.5-5.75):

Add 7.91g to 50 mL DI water and then 0.9g pellets NaOH. Adjust pH with 10M NaOH/HCl. Filter sterilize and gas with N₂.

Vitamin solution

Biotin.....	20 mg
Folic acid.....	20 mg
Pyridoxine HCl.....	100 mg
Thiamine HCl.....	50 mg
Nicotinic acid.....	50 mg
DL-calcium pantothenate.....	50 mg
Vitamin B12.....	1.0 mg
p-aminobenzoic acid.....	50 mg
lipoic acid.....	50 mg
DI Water.....	1000 ml

Filter sterilize and keep at 4C in dark.

2% YE

Yeast Extract.....1.0 g

DI Water.....50 mL

Filter sterilize and gas with N₂.

0.05 M CoM (2 Mercaptoethanesulfonic acid)

Add 0.411g to 50 mL DI water, gas and autoclave.

0.02M Acetate

Add 0.136g to 50mL DI water, filter sterilize and gas with N₂