

ATCC medium: 2010 *Chromatium* medium (ATCC medium 37) with 3% NaCl

ATCC Medium 37 (see below) with 3% NaCl

ATCC Medium 37:

Solution 1:

CaCl₂2.0 g
Distilled water.....2.5 L

Distribute 2000 ml in 75-80 ml amounts into 127 ml (4 oz) screw-capped Boston round bottles and autoclave.

Solution 2:

Heavy Metal Solution (see below).....50.0 ml
Vitamin B12 Solution (see below).....3.0 ml
Vitamin Solution (see below).....15.0 ml
KH₂PO₄1.0 g
KCl.....1.0 g
NH₄Cl0.8 g
MgCl₂ . 6H₂O0.8 g
Sodium ascorbate*.....2.4 g
Distilled water.....32.0 ml

*May be omitted if large inocula are used.

Solution 3:

NaHCO₃4.5 g
Distilled water.....900.0 ml

Bubble gaseous CO₂ through for at least 30 minutes. After CO₂ saturation of Solution 3, add Solution 2 and immediately filter-sterilize using CO₂ pressure to push the liquid through (no suction). Add this sterile filtered solution to the 127 ml bottles containing 75-80 ml of Solution 1 so that it completely fills the bottle.

Solution 4:

Na₂S . 9H₂O3.0 g
Distilled water.....200.0 ml

Autoclave with a magnetic-stirrer rod in the flask. Partially neutralize the sterilized Solution 4 by adding, on a magnetic stirrer, drop by drop, 2.0 ml sterile 2 M H₂SO₄.

Directions for completion of Medium:

Remove 6 ml of medium in the Boston round bottles that were filled to full capacity as directed under Solutions 2 and 3. Replace this volume with an equal volume of neutralized Solution 4. Allow the bottles to stand at least overnight to develop a hazy, white precipitate before inoculating. At this time, the final pH will be 6.7 +/- 0.1. To inoculate, remove 6 ml of completed medium and replace it with an equal volume of inoculum. Grow cultures under tungsten light.

Heavy Metal Solution:

EDTA (should be dissolved first).....	1.5 g
Modified Hoagland Trace Element Solution (see below).....	6.0 ml
FeSO ₄ . 7H ₂ O	0.2 g
ZnSO ₄ . 7H ₂ O	0.1 g
MnCl ₂ . 4H ₂ O	0.02 g
Distilled water.....	1.0 L

Vitamin B12 Solution:

Vitamin B12 (Cyanocobalamine).....	2.0 mg
Distilled water.....	100.0 ml

Vitamin Solution:

Biotin.....	0.2 mg
Nicotinic acid.....	2.0 mg
Thiamine.....	1.0 mg
p-Aminobenzoic acid.....	0.1 mg
Pantothenic acid.....	0.5 mg
Pyridoxamine 2HCl.....	5.0 mg
Distilled water.....	100.0 ml

Modified Hoagland Trace Element Solution:

AlCl ₃	1.0 g
KI	1.0 g
KBr	0.5 g
LiCl	0.5 g
MnCl ₂ . 4H ₂ O	7.0 g
H ₃ BO ₃	11.0 g
ZnCl ₂	1.0 g
CuCl ₂	1.0 g
NiCl ₂	1.0 g
CoCl ₂	1.0 g
SnCl ₂ . 2H ₂ O	0.5 g
BaCl ₂	0.5 g
Na ₂ MoO ₄	0.5 g
NaVO ₃ . H ₂ O	0.1 g
Na ₂ SeO ₃	0.5 g

Dissolve each salt in distilled water before mixing. Adjust the pH of each solution to below 7.0. Adjust the final pH to 3-4. The total final volume is 3.6 L. The flaky yellow precipitate which is formed after mixing transforms after a few days into a very fine white precipitate. Mix the solution thoroughly before use.