

ATCC medium: 2094 AN 1 medium

K₂HPO₄1.5 g
MgCl₂ . 6H₂O0.3 g
NaCl.....2.5 g
Wolfe's Vitamins (see below).....1.0 ml
Modified Hoagland Trace Elements (see below)...0.3 ml
Tryptone.....8.0 g
Sodium thioglycollate.....0.5 g
Distilled water.....1.0 L
Sulfur*.....~2 gr

*Prepare sterilized sulfur powder using dry heat sterilization prior to preparation of medium. Aseptically add a small amount of sterile sulfur powder to dry sterile Hungate tubes prior to dispensing medium.

Combine ingredients except for vitamins and sulfur. Autoclave at 121C for 15 minutes. After autoclaving, gas with nitrogen. When cool, adjust to pH 7.4. Add filter-sterilized vitamin solution. Under nitrogen, dispense into the Hungate tubes containing the sulfur powder. Dispense in 5-6 ml amounts. Use thick stoppers.

Wolfe's Vitamin Solution:

Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).

Biotin.....2.0 mg
Folic acid.....2.0 mg
Pyridoxine hydrochloride....10.0 mg
Thiamine . HCl.....5.0 mg
Riboflavin.....5.0 mg
Nicotinic acid.....5.0 mg
Calcium D-(+)-pantothenate...5.0 mg
Vitamin B12.....0.1 mg
p-Aminobenzoic acid.....5.0 mg
Thioctic acid.....5.0 mg
Distilled water.....1.0 L

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.