

ATCC medium: 1045 Methanobacteria medium

Mineral Solution 1:

K₂HPO₄6.0 g
Distilled water.....1.0 L

Mineral Solution 2:

KH₂PO₄6.0 g
(NH₄)₂SO₄.....6.0 g
NaCl.....12.0 g
MgSO₄ . 7H₂O2.4 g
CaCl₂ . 2H₂O1.6 g
Distilled water.....1.0 L

Sodium Carbonate Solution:

Na₂CO₃8.0 g
Distilled water.....100.0 ml

Reducing Agent:

Add 300 mg L-Cysteine . HCl to 10 ml of water and 300 mg Na₂S . 9H₂O to a second 10 ml of water: tube under nitrogen gas. After autoclaving at 121C for 15 minutes under fast exhaust and allowing to cool, mix equal amounts of the cysteine and the sodium sulfide (under nitrogen). The mixture should be clear and can be kept for up to two weeks.

Prepare the complete medium by mixing the above components in the following proportions:

83.5 parts of distilled water
5.0 parts of Mineral Solution 2
2.5 parts of Mineral Solution 1
5.0 parts of 8.0% Na₂CO₃ Solution
Agar (if needed).....20.0 g
1.0 part of Wolfe's Mineral Solution (see below)
1.0 part of Wolfe's Vitamin Solution (see below)
2.0 parts of Cysteine-Sulfide Reducing Agent
Resazurin (0.025% solution).....4.0 ml/L

Mix all the ingredients except the Wolfe's Vitamin and Cysteine/Na₂S Solutions. After autoclaving for 15 minutes, cool under 80% N₂, 20% CO₂ and add the sterile Wolfe's Vitamin Solution, then Cysteine/Na₂S Solution. Adjust pH to 7.2 if necessary and tube anaerobically, aseptically.

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid.....	1.5 g
MgSO ₄ . 7H ₂ O	3.0 g
MnSO ₄ . H ₂ O	0.5 g
NaCl.....	1.0 g
FeSO ₄ . 7H ₂ O	0.1 g
CoCl ₂ . 6H ₂ O	0.1 g
CaCl ₂	0.1 g
ZnSO ₄ . 7H ₂ O	0.1 g
CuSO ₄ . 5H ₂ O	0.01 g
AlK(SO ₄) ₂ . 12H ₂ O.....	0.01 g
H ₃ BO ₃	0.01 g
Na ₂ MoO ₄ . 2H ₂ O.....	0.01 g
Distilled water.....	1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

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

Filter-sterilize.