

ATCC medium: 2635 *Desulfuromonas michiganensis* medium

Salt solution (see below).....	10.00 ml
Wolfe's Mineral Solution (see below).....	1.00 ml
Wolfe's Vitamin Solution (see below).....	10.00 ml
Se/Wo solution (see below).....	1.00 ml
Resazurin.....	0.50 mg
Sodium acetate.....	0.50 g
Cysteine-HCl. 5H ₂ O.....	0.05 g
Na ₂ S . 9H ₂ O.....	0.05 g
NaHCO ₃	2.50 g
Distilled water.....	1.00 L

Prepare the medium anaerobically under a steady stream of 80% N₂ and 20% CO₂. Adjust the pH before autoclaving to 7.2. Filter sterilize anaerobically a stock solutions of Fumarate (1.0M) and Wolfe's Vitamin Solution. Prepare a stock solution of Tetrachloroethene (See Below). Autoclave the base medium at 121°C for 15 minutes. After cooling, aseptically and anaerobically add 10ml of the Wolfe's vitamin solution. Dispense the medium anaerobically into rubber stopper test tubes. The night before inoculation, add 200µl of PCE per 100ml of medium and 100µl of Fumarate per 10ml of medium.

PCE (Tetrachloroethene) solution:

Prepare the PCE stock solution in hexadecane (10ul PCE in 100ul Hexadecane). Use only Teflon-lined rubber stoppers due to absorption of the PCE. Autoclave the solution

Salt Solution (100X):

NaCl.....	100.0 g
MgCl ₂ . 6H ₂ O.....	50.0 g
NH ₄ Cl.....	30.0 g
KH ₂ PO ₄	20.0 g
KCl.....	30.0 g
CaCl ₂ . 2H ₂ O.....	1.5 g

Wolfe's Mineral Solution:

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

Nitritotriacetic acid.....	1.500 g
MgSO ₄ . 7H ₂ O.....	3.000 g
MnSO ₄ . H ₂ O.....	0.500 g

NaCl.....	1.000	g
FeSO ₄ . 7H ₂ O	0.100	g
Co(NO ₃) ₂ . 6H ₂ O.....	0.100	g
CaCl ₂	0.100	g
ZnSO ₄ . 7H ₂ O	0.100	g
CuSO ₄ . 5H ₂ O	0.010	g
AlK(SO ₄) ₂ . 12H ₂ O.....	0.010	g
H ₃ BO ₃	0.010	g
Na ₂ MoO ₄ . 2H ₂ O.....	0.010	g
Na ₂ SeO ₃ (anhydrous)	0.001	g
Na ₂ WO ₄ . 2H ₂ O.....	0.010	g
NiCl ₂ . 6H ₂ O	0.020	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

Se/Wo Solution (Per/L):

Na ₂ SeO ₃ . 5H ₂ O.....	6.0	mg
Na ₂ WO ₄ . 2H ₂ O.....	8.0	mg
NaOH.....	0.5	g