

ATCC medium: 1601 *Desulfonema limicola* medium

Solution A:

Na ₂ SO ₄	3.0 g
NaCl.....	13.0 g
MgCl ₂ . 6H ₂ O	2.2 g
CaCl ₂ . 2H ₂ O	0.15 g
KCl.....	0.5 g
KH ₂ PO ₄	0.2 g
NH ₄ Cl	0.3 g
Resazurin.....	0.5 mg
Distilled water.....	850.0 ml

Solution B:

Trace Element Solution SL-10 (see below).....	1.0 ml
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Solution C:

NaHCO ₃	5.0 g
Distilled water.....	100.0 ml

Solution D:

Sodium acetate . 3H ₂ O	2.5 g
Distilled water.....	10.0 ml

Solution E:

Disodium succinate.....	0.1 g
Distilled water.....	1.0 ml

Solution F:

Wolfe's Vitamin Solution (see below).....	10.0 ml
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Solution G:

Artificial sediment:

AlCl ₃ . 6H ₂ O [4.9% (w/v)]	5.0 ml
Na ₂ CO ₃ [10.6% (w/v)]	1.6 ml

Solution H:

Rumen fluid, clarified.....	20.0 ml
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Solution I:

Na ₂ S . 9H ₂ O	0.4 g
Distilled water.....	10.0 ml

Directions for complete medium:

Boil Solution A for a few minutes, cool to room temperature, gas with 80% N₂, 20% CO₂ gas mixture to reach a pH of 6.0, then autoclave anaerobically under the same gas mixture. Autoclave Solutions B, D, E, G, H, and I separately under nitrogen. Filter-sterilize Solution C and flush with 80% N₂, 20% CO₂ to remove dissolved oxygen. Filter-sterilize Solution F and outgas with N₂. Add Solutions B through I to the sterile cooled Solution A in the sequence indicated. Distribute the completed medium anaerobically under 80% N₂, 20% CO₂ into appropriate vessels. Final pH of the medium should be 7.6.

Addition of 10-20 mg/L sodium dithionite (e.g., from 5% (w/v) solution freshly prepared under N₂ and filter-sterilized) just before inoculation may stimulate growth at the beginning. For transfers use 5-10% inoculum. According to F. Widdel, cultures of *Desulfohalobium* are usually grown in media containing an artificial light sediment of aluminum phosphate. Alternatively, a viscous medium prepared with 1-2 g/L washed agar can be used (Widdel et al., Arch. Microbiol. 134:286-294, 1983):

Wash Agar (BD) three times for 1 hour each time with distilled water at room temperature. Finally, suspend 20.0 g agar/L distilled water and autoclave. Add 50 ml of the hot agar to the hot sterile medium part A (when preparing part A calculated for 1.0 L, subtract the volume of the agar solution). After cooling to room temperature complete the medium by adding parts B through I. For mass cultures, use the aluminum phosphate as artificial sediment.

Trace Elements Solution SL-10:

HCl (25%).....	10.0 ml
FeCl ₂ . 4H ₂ O	1.5 g
ZnCl ₂	70.0 mg
MnCl ₂ . 4H ₂ O	100.0 mg
H ₃ BO ₃	6.0 mg
CoCl ₂ . 6H ₂ O	190.0 mg
CuCl ₂ . 2H ₂ O	2.0 mg
NiCl ₂ . 6H ₂ O	24.0 mg
Na ₂ MoO ₄ . 2H ₂ O.....	36.0 mg
Distilled water.....	990.0 ml

Dissolve FeCl₂ in the HCl, dilute with water, add and dissolve the other salts; adjust pH to 6.0 with NaOH, and fill to 1.0 L with distilled water.

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