

ATCC medium: 1647 *Desulfobacterium indolicum* medium

Solution A:

Na ₂ SO ₄	3.0 g
KH ₂ PO ₄	0.2 g
NH ₄ Cl	0.3 g
NaCl	21.0 g
MgCl ₂ . 6H ₂ O	3.0 g
KCl	0.5 g
CaCl ₂ . 2H ₂ O	0.15 g
Resazurin	1.0 mg
Distilled water	900.0 ml

Solution B:

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

Solution C:

NaHCO ₃	2.5 g
Distilled water	50.0 ml

Solution D:

Indole Solution (see below)	30.0 ml
-----------------------------------	---------

Solution E:

Wolfe's Vitamin Solution (see below)	10.0 ml
--	---------

Solution F:

Na ₂ SeO ₃ . 5H ₂ O	3.0 mg
0.01 M NaOH	1.0 L

Use 1.0 ml/L medium.

Solution G:

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

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.

Indole Solution:

Dissolve 0.3 g of indole in 100 ml water by heating and shaking in a closed bottle under nitrogen gas, autoclave, then add sterile anaerobic 30% NaCl solution (7 ml) and 40% MgCl₂ · 6H₂O solution (0.7 ml). Store the indole solution in the dark. Reheat and shake before use. Add 30 ml/L medium at the beginning of incubation, and the same amount two more times later in the growth cycle.

Directions for complete medium:

Prepare and autoclave Solution A anaerobically under 80% N₂, CO₂. Filter-sterilize and gas Solution C for 20 minutes with 80% N₂, CO₂. Filter-sterilize Solutions B, E and F and gas with N₂. Autoclave Solution G under N₂. Add Solutions B, C, E, F and G to the sterile, cooled Solution A in the sequence indicated. Add Solution D prior to inoculation. Final pH of the medium should be 7.0. Addition of 10-20 mg/L sodium dithionite (e.g., from 5% (w/v) solution freshly prepared under N₂ and filter-sterilized) may stimulate growth of all strains at the beginning. For transfers use 5-10% inoculum. Incubate all strains in the dark.

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