

ATCC medium: 2309 Anaerobic oxalate medium

Solution A:

Na ₂ SO ₄	3.0 g
KH ₂ PO ₄	0.2 g
NH ₄ Cl	0.3 g
NaCl	7.0 g
MgCl ₂ . 6H ₂ O	1.3 g
KCl	0.5 g
CaCl ₂ . 2H ₂ O	0.15 g
Yeast extract	1.0 g
Resazurin	1.0 mg
Distilled water	870.0 ml

Boil solution for a few minutes, cool to room temperature, gas with 80% N₂, 20% CO₂ to reach a pH below 6.0. Autoclave at 121C for 15 minutes under the same gas mixture.

Solution B:

Trace Elements SL-10 (see below) ..10.0 ml

Autoclave at 121C for 15 minutes under oxygen-free nitrogen.

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.

Solution C:

NaHCO ₃	5.0 g
Distilled water	100.0 ml

Filter-sterilize and flush with 80% N₂, 20% CO₂ to remove dissolved oxygen.

Solution D:

Sodium acetate . 3H₂O.....0.25 g
Distilled water.....10.0 ml

Autoclave at 121C for 15 minutes under oxygen-free nitrogen.

Solution E:

Vitamin Solution (see below).....10.0 ml

Autoclave at 121C for 15 minutes under oxygen-free nitrogen.

Vitamin Solution:

Biotin.....2.0 mg
Folic acid.....2.0 mg
Pyridoxine-HCl.....10.0 mg
Thiamine-HCl . 2H₂O.....5.0 mg
Riboflavin.....5.0 mg
Nicotinic acid.....5.0 mg
D-Calcium-pantothenate.....5.0 mg
Vitamin B12.....0.1 mg
p-Aminobenzoic acid.....5.0 mg
Lipoic acid.....5.0 mg
Distilled water.....1.0 L

Solution F:

Na₂S . 9H₂O0.4 g
Distilled water.....10.0 ml

Autoclave at 121C for 15 minutes under oxygen-free nitrogen.

Complete Medium:

Add solutions B through F to the sterile, cooled Solution A in the sequence given. Distribute anaerobically under 80% N₂, 20% CO₂. Final pH of the medium should be 7.1-7.4.

After the medium is dispensed, add ammonium oxalate (400 mM stock solution) to a final concentration of 30-40 mM. The addition of 10-20 mg of sodium dithionite per liter of medium (from 5% w/v solution, freshly prepared under N₂ and filter-sterilized) may stimulate initial growth.