

ATCC Medium: 2823 Modified Methylophaga Agar/Broth Medium

Complete Medium

Mineral Base (see below).....	100.0 ml
Solution T (see below).....	2.0 ml
Vitamin B12 (0.1 mcg/ml) filter-sterilized.....	1.0 ml
Methanol (filter-sterilized).....	10.0 ml

Aseptically combine each solution.

For solid medium prepare a 2X Mineral Base solution and a 3% agar solution and autoclave both separately. Cool to 50C and aseptically mix the solutions in equal volume before adding solution T, the B12 solution and methanol.

Mineral Base

NaCl.....	24.0 g
MgCl ₂ x 6H ₂ O.....	3.0 g
MgSO ₄ x 7H ₂ O.....	2.0 g
KCl.....	0.5 g
CaCl ₂ x 2H ₂ O.....	1.0 g
Bis-Tris.....	0.5 g
Wolfe's Mineral Solution (see below).....	10.0 ml
DI Water.....	990.0 ml

Adjust to pH 7.3 with HCl. Autoclave at 121C.

Solution T

KH ₂ PO ₄	0.7 g
NH ₄ Cl.....	10.0 g
Bis-Tris.....	10.0 g
Ferric ammonium citrate.....	0.3 g
DI Water.....	100.0 ml

Adjust to pH 7.3. Autoclave at 121C.

Wolfe's Mineral Solution

Nitriloacetic Acid.....	1.5 g
MgSO ₄	3.0 g
MnSO ₄	0.5 g
NaCl.....	1.0 g
FeSO ₄	0.1 g
CaCl ₂	0.1 g
CoCl ₂	0.1 g
ZnSO ₄	0.1 g
CuSO ₄	0.01 g

$\text{AlK}(\text{SO}_4)_2$	0.01 g
H_3BO_4	0.01 g
Na_2MoO_4	0.01 g
DI Water.....	1000 ml