

ATCC medium: 2099 *Hyphomicrobium* DMF medium

MgSO ₄ . 7H ₂ O	0.1 g
(NH ₄) ₂ SO ₄	0.26 g
KNO ₃	0.30 g
HEPES buffer.....	2.38 g
K ₂ HPO ₄	0.52 g
CaCl ₂ Stock Solution (see below)	1.0 ml
FeSO ₄ /EDTA Stock Solution (see below)	1.0 ml
Trace Mineral Stock Solution (see below)	1.0 ml
N,N-dimethylformamide.....	15.0 ml
Distilled water.....	985.0 ml

Mix all ingredients except FeSO₄/EDTA Stock Solution and dimethylformamide. Autoclave at 121C for 15 minutes. Aseptically add FeSO₄/EDTA Stock and dimethylformamide. Adjust pH to 7.5.

CaCl₂ Stock Solution:

CaCl ₂ . 2H ₂ O	7.35 g
Distilled water.....	1.0 L

FeSO₄/EDTA Stock Solution:

FeSO ₄ . 7H ₂ O	0.556 g
EDTA.....	0.744 g
Distilled water.....	100.0 ml

Autoclave at 121C for 10 minutes. The resulting solution should be a clear medium yellow. Store at 4C. A precipitate may form after several months. If precipitate remains after warming, discard the solution.

Trace Mineral Stock Solution:

CuSO ₄ . 5H ₂ O	8.0 mg
ZnSO ₄ . 7H ₂ O	10.0 mg
NiCl ₂ . 6H ₂ O	10.0 mg
Co(NO ₃) ₂ . 6H ₂ O.....	14.5 mg
KI.....	3.0 mg
MnSO ₄ . H ₂ O	8.4 mg
Na ₂ MoO ₄ . 2H ₂ O.....	9.7 mg
EDTA.....	0.102 g
Distilled water.....	100.0 ml

Add EDTA to water first. Then add metals one at a time, mixing well after each addition.