

**ATCC medium: 2197 *Filobacillus* medium**

Peptone.....	0.75 g
Yeast extract.....	0.75 g
Glucose.....	0.75 g
NaCl.....	100.0 g
Modified Hutner's Basal Salts (see below).....	20.0 ml
Wolfe's Vitamin Solution (see below).....	20.0 ml
0.1 M Tris/HCl buffer (pH 7.5).....	50.0 ml
Artificial Seawater (see below).....	250.0, ml
Distilled water.....	660.0 ml

For solid medium, use 18 grams of agar and adjust pH of Tris/HCl buffer to 8.5.

Autoclave at 121C for 15 minutes.

For artificial seawater, use "Instant Ocean" (32 g/L) or the Lyman and Fleming solution below.

*Modified Hutner's Basal Salts:*

Nitritotriacetic acid.....	10.0 g
MgSO <sub>4</sub> . 7H <sub>2</sub> O .....	29.7 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	3.34 g
Ammonium molybdate.....	9.25 mg
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	99.0 mg
Metals "44" (see below).....	50.0 ml
Distilled water to.....	1.0 L

Dissolve and neutralize the nitritotriacetic acid with KOH (7.3 g); add the other ingredients and adjust the pH to 6.6 - 6.8 before bringing the volume to 1.0 L with distilled water.

*Metals "44":*

EDTA.....	0.25 g
ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....	1.1 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.5 g
MnSO <sub>4</sub> . 7H <sub>2</sub> O .....	0.154 g
CuSO <sub>4</sub> . 5H <sub>2</sub> O .....	0.04 g
Co(NO <sub>3</sub> ) <sub>2</sub> . 6H <sub>2</sub> O.....	0.025 g
Na <sub>2</sub> B <sub>4</sub> O <sub>7</sub> . 10H <sub>2</sub> O .....	0.018 g
Distilled water.....	100.0 ml

Initially add a few drops of H<sub>2</sub>SO<sub>4</sub> to the distilled water to retard precipitation.

*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

*Artificial Seawater (Lyman and Fleming, 1940):*

NaCl.....	23.47 g
MgCl <sub>2</sub> .....	4.98 g
Na <sub>2</sub> SO <sub>4</sub> .....	3.92 g
CaCl <sub>2</sub> .....	1.10 g
KCl.....	0.66 g
NaHCO <sub>3</sub> .....	0.19 g
KBr.....	0.10 g
H <sub>3</sub> BO <sub>3</sub> .....	0.026 g
SrCl <sub>2</sub> .....	0.024 g
NaF.....	0.003 g
Distilled water.....	1.0 L