

**ATCC medium: 1398 Modified low phosphate buffered basal medium**

Yeast extract (autoclaved as separate solution).....	1.0 g
Pectin.....	4.0 g
NH <sub>4</sub> Cl.....	1.0 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O.....	0.2 g
KH <sub>2</sub> PO <sub>4</sub> .....	0.3 g
Na <sub>2</sub> HPO <sub>4</sub> .....	0.72 g
Trace Minerals (see below).....	10.0 ml
2.5% FeSO <sub>4</sub> . 7H <sub>2</sub> O.....	0.03 ml
0.2% Resazurin.....	1.0 ml
Wolfe's Vitamin Solution (see below).....	5.0 ml
Reducing Agent (see below), added after sterilization.....	20.0 ml
Distilled water.....	1.0 L

Adjust final pH to 7.3, +/- 0.1, with Na<sub>2</sub>CO<sub>3</sub>/HCl. Boil medium and cool under 90% N<sub>2</sub>, 10% CO<sub>2</sub>. Dispense medium anaerobically using the same gas mixture. For destruction of possible indigenous thermophilic spores, autoclave basal medium, yeast extract and reducing agent for 45 minutes at 121C. Aseptically add yeast extract to cooled basal medium to a final concentration of 0.1%.

Add reducing agent just prior to inoculation.

*Trace Minerals:*

Nitrilotriacetic acid (adjusted to pH 6.5 with KOH).....	12.8 g
FeSO <sub>4</sub> . 7H <sub>2</sub> O.....	0.1 g
MnCl <sub>2</sub> . 4H <sub>2</sub> O.....	0.1 g
CoCl <sub>2</sub> . 6H <sub>2</sub> O.....	0.16 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O.....	0.1 g
ZnCl <sub>2</sub> .....	0.1 g
CuCl <sub>2</sub> .....	0.02 g
H <sub>3</sub> BO <sub>3</sub> .....	0.01 g
Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O.....	0.01 g
NaCl.....	1.0 g
NiSO <sub>4</sub> . 6H <sub>2</sub> O.....	26.0 mg
Na <sub>2</sub> SeO <sub>3</sub> .....	0.02 g
Distilled water.....	1.0 L

*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

*Reducing Agent:*

2.5% Na<sub>2</sub>S . 9H<sub>2</sub>O. Purge freshly prepared solution with N<sub>2</sub> gas for 20 minutes, stopper with butyl rubber under N<sub>2</sub>, and autoclave for 45 minutes at 121C. Aseptically add 0.12 ml reducing agent to 6.0 ml medium.