

**ATCC medium: 2252 *Bacillus subterraneus* medium (MR medium)**

NaHCO <sub>3</sub> .....	3.6 g
NH <sub>3</sub> Cl .....	1.0 g
K <sub>2</sub> HPO <sub>4</sub> . 3H <sub>2</sub> O.....	0.08 g
MgCl <sub>2</sub> . 6H <sub>2</sub> O .....	4.5 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.375 g
NaCl.....	32.0 g
Yeast extract.....	2.0 g
Wolfe's Vitamin Solution (see below) .....	10.0 ml
Zeikus Trace Elements (see below) .....	1.0 ml
Distilled water.....	1.0 L

Combine all ingredients except the NaHCO<sub>3</sub>; boil for 15 minutes. Cool under N<sub>2</sub> gas. After cooling, add NaHCO<sub>3</sub> and adjust pH to 7.1. Dispense anaerobically in 10-ml aliquots.

Prepare 15 ml of 2 M glucose. As a separate solution, prepare 15 ml of 2 M sodium nitrate. Sterilize both solutions. Prior to inoculation, add glucose and nitrate solutions to a final concentration of 20-mM of each to act as electron donors and acceptors.

*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

*Zeikus Trace Elements:*

Nitriilotriacetic acid.....	12.8 g
COCl <sub>2</sub> . 6H <sub>2</sub> O .....	0.17 g
CaCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.1 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
NaCl.....	0.1 g
ZnCl <sub>2</sub> .....	0.1 g
NiSO <sub>4</sub> . 6H <sub>2</sub> O .....	0.026 g
CuCl <sub>2</sub> . 2H <sub>2</sub> O .....	0.2 g
Na <sub>2</sub> SeO <sub>3</sub> .....	0.017 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
Distilled water.....	1.0 L