ATCC medium: 2473 *Theroanaerobacter* medium

$NaHCO_3$ .....2.5 g
$CaCl_2$. H_2O .....0.08 g
NH_4Cl.....1.0 g
$MgCl_2$. $6H_2O$ .....0.2 g
NaCl.....10.0 g
MOPS.....2.5 g
Resazurin (0.01%).....1.0 g
Yeast extract.....0.5 g
Wolfe's Mineral Solution (see below)....10.0 ml
Wolfe's Vitamin Solution (see below).... 1.0 ml
Distilled water.....1.0 L

Adjust to pH 7.3. Bring to a boil and cool with N_2.
Dispense into Hungate tubes and autoclave at 121C for 15 minutes.

Stock solutions of 1M Ferric Citrate(Sigma F-6129) and 2M Glucose should be made anaerobically and filter sterilized. Just before inoculation 150 µl of Ferric Citrate and 100 µl of Glucose should be added per 10.0 ml of medium. This should be done anaerobically.

Wolfe's Mineral Solution:
Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS).
Nitrilotriacetic acid.....1.5 g
$MgSO_4$. $7H_2O$ .....3.0 g
$MnSO_4$. H_2O .....0.5 g
NaCl.....1.0 g
$FeSO_4$. $7H_2O$ .....0.1 g
$CoCl_2$. $6H_2O$ .....0.1 g
$CaCl_2$.....0.1 g
$ZnSO_4$. $7H_2O$ .....0.1 g
$CuSO_4$. $5H_2O$ .....0.01 g
$AlK(SO_4)_2$. $12H_2O$.....0.01 g
H_3BO_3 .....0.01 g
Na_2MoO_4 . $2H_2O$.....0.01 g
Distilled water.....1.0 L

Add nitrilotriacetic acid to approximately 500 ml of water and adjust to pH 6.5 with KOH to dissolve the compound. Bring volume to 1.0 L with remaining water and add remaining compounds one at a time.

Wolfe's Vitamins:
Available from ATCC as a sterile ready-to-use liquid (Vitamin Supplement, catalog no. MD-VS).
Folic acid.....2.0 mg
Pyridoxine hydrochloride....10.0 mg
Riboflavin.....5.0 mg
Biotin.....2.0 mg
Thiamine.....5.0 mg
Nicotinic acid.....5.0 mg
Pantothenic acid.....5.0 mg
Vitamin B12.....0.1 mg
p-Aminobenzoic acid.....5.0 mg
Thioctic acid.....5.0 mg
Distilled water.....1.0 L