ATCC medium: 1279 *Haloanaerobium praevalens* medium (ATCC medium 1043) with NaCl at a concentration of 13%

ATCC Medium 1043 (see below) with NaCl at a concentration of 13%

ATCC Medium 1043:

K_2HPO_4	348.0 mg
KH_2PO_4	227.0 mg
NH_4Cl	500.0 mg
$MgSO_4 \cdot 7H_2O$	500.0 mg
$CaCl_2 \cdot 2H_2O$	250.0 mg
$FeSO_4 \cdot 7H_2O$	2.0 mg
Trace Elements Solution SL-6 (see below)	3.0 ml
Wolfe's Vitamin Solution (see below)	10.0 ml
Yeast Extract (BD 212750)	2.0 g
Casitone (BD 225930)	2.0 g
NaCl	2.25 g
Resazurin (0.025% solution)	4.0 ml
$NaHCO_3$ Solution (see below)	20.0 ml
Methanol	10.0 ml
Reducing Agent (see below)	20.0 ml
Agar	20.0 g
Distilled water	950.0 ml

Mix all of the ingredients except the Wolfe's Vitamin Solution, $NaHCO_3$ Solution, Methanol and Cysteine/Na_2S Solution (Reducing Agent) and autoclave for 15 minutes. After autoclaving, cool under 80% N_2, 20% CO_2 and add the solutions in the order listed above. Adjust pH to 6.8 if necessary and tube anaerobically and aseptically.

Trace Elements Solution SL-6:

$ZnSO_4 \cdot 7H_2O$	0.10 g
$MnCl_2 \cdot 4H_2O$	0.03 g
H_3BO_3	0.3 g
$CoCl_2 \cdot 6H_2O$	0.2 g
$CuCl_2 \cdot 2H_2O$	0.01 g
$NiCl_2 \cdot 6H_2O$	0.02 g
$Na_2MoO_4 \cdot 2H_2O$	0.03 g
Distilled water	1.0 L

Adjust final pH of Trace Elements Solution SL-6 to 3.4.

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

$NaHCO_3$ Solution:
Dissolve 850 mg of $NaHCO_3$ in 20.0 ml of water. After filter-sterilizing, equilibrate under CO_2 for 20 minutes.

Reducing Agent:
Add 300 mg L-cysteine . HCl to 10 ml of water and 300 mg Na_2S . $9H_2O$ to a second 10 ml of water; tube under nitrogen gas. After autoclaving at 121C for 15 minutes under fast exhaust and allowing to cool, mix equal amounts of the cysteine and the sodium sulfide (under nitrogen). The mixture should be clear and can be used up to two weeks.