ATCC Medium 2632: <u>Modified *Thermotoga elfii* medium</u>

NH_4Cl	1.0 g
K_2HPO_4	0.3 g
KH_2PO_4	0.3 g
$MgCl_2 \cdot 6H_2O$	0.2 g
$CaCl_2 \cdot 2H_2O$	0.1 g
NaCl	10.0 g
KCl	0.1 g
L-Cysteine · HCl	0.5 g
Sodium acetate	0.5 g
Yeast extract	5.0 g
Trypticase Peptone (BD 211921)	5.0 g
Wolfe's Mineral Solution (see below)	10.0 mL
Resazurin	0.5 mg
Cysteine-HCl · $5H_2O$	0.5 mg
Na_2CO_3	2.0 g
$Na_2S_2O_3 \cdot 9H2O$	5.0 g
Glucose	4.0 g
$Na_2S \cdot 9H_2O$	0.5 g
Distilled water	1.0 L

Prepare the medium anaerobically under a steady stream of 80% N_2 and 20% CO_2. Autoclave separately and anaerobically stock solutions of Na2CO3, thiosulfate, glucose and sodium sulfide.

The pH of the completed medium is 7.5.

Wolfe's Mineral Solution:

Available from ATCC as a sterile ready-to-use liquid (Trace Mineral Supplement, catalog no. MD-TMS.)

Nitrilotriacetic acid…… 1.50 g
$MgSO_4 \cdot 7H_2O$…….…….. 3.00 g
$MnSO_4 \cdot H_2O$…….. 0.50 g
NaCl…….. 1.00 g
$FeSO_4 \cdot 7H_2O$……. 0.10 g
$Co(NO_3)_2 \cdot 6H_2O$…..……... 0.10 g
$CaCl_2$ .....…….……... 0.10 g
$ZnSO_4 \cdot 7H_2O$.....…….……... 0.10 g
$CuSO_4 \cdot 5H_2O$…..…… 0.01 g
$AlK(SO_4)_2 \cdot 12H_2O$…..…… 0.01 g
H_3BO_3…..…… 0.01 g
$Na_2MoO_4 \cdot 2H_2O$…..……... 0.01 g
Na_2SeO_3 (anhydrous) ……... 0.001 g
$NA_2WO_4 \cdot 2H_2O$…..…… 0.01 g
$NiCl_2 \cdot 6H_2O$…..…….. 0.02 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.