**ATCC medium: 1302 Halophilic *Clostridium* medium**

*Solution 1:*

| | |
|---|---|
| NaCl | 105.0 g |
| KCl | 7.5 g |
| L-glutamic acid (dissolves upon heating of the final mixture) | 4.0 g |
| Yeast extract | 2.0 g |
| Nutrient broth | 2.0 g |
| Casamino acids | 2.0 g |
| $FeSO_4 \cdot 7H_2O$ | 2.0 mg |
| Resazurin | 1.0 mg |
| Wolfe's Vitamin Solution (see below) | 10.0 ml |
| Wolfe's Mineral Solution (see below) | 10.0 ml |
| 2.5 N NaOH | 12.5 ml |
| Distilled water | 1.0 L |

*Solution 2:*

| | |
|---|---|
| $MgCl_2 \cdot 6H_2O$ | 20.3 g |
| $CaCl_2 \cdot 2H_2O$ | 7.35 g |
| Distilled water | 100.0 ml |

Dissolve the ingredients in water. Boil Solution 1 under nitrogen until the reasurin turns red, then add 0.5 g L-cysteine . HCl, and continue boiling under nitrogen until the medium is yellow. (The final volume should be around 900 ml.) Using anaerobic techniques, dispense the medium in 9 ml portions into stoppered serum tubes, cap and autoclave.

Bubble Solution 2 with nitrogen and autoclave. Add 1 ml to each tube of the autoclaved Solution 1. Adjust pH if necessary to 6.2-7.0 using sterile gassed NaOH or HCl.

Note:
For solid medium add to Solution 1: 2.0 g soluble starch, 20.0 g Agar, Bacto (BD 214050), and 5.0 g $CaCO_3$, the last compound being added after complete dissolution of the glutamic acid in the presence of the NaOH. Autoclave and add Solution 2 as instructed above. In the presence of $CaCO_3$, the final pH may be higher than 7.0, but there is no need to adjust it.

*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 |

*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 |
| $MgSO_4 \cdot 7H_2O$ | 3.0 g |
| $MnSO_4 \cdot H_2O$ | 0.5 g |
| NaCl | 1.0 g |
| $FeSO_4 \cdot 7H_2O$ | 0.1 g |
| $CoCl_2 \cdot 6H_2O$ | 0.1 g |
| $CaCl_2$ | 0.1 g |
| $ZnSO_4 \cdot 7H_2O$ | 0.1 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 |
| 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.