

**ATCC medium: 1824 *Halobacteroides* medium**

|   |         |
|---|---------|
| NH <sub>4</sub> Cl .....                    | 0.33 g  |
| KH <sub>2</sub> PO <sub>4</sub> .....       | 0.33 g  |
| KCl.....                                    | 0.33 g  |
| MgCl <sub>2</sub> . 6H <sub>2</sub> O ..... | 0.33 g  |
| NaCl.....                                   | 150.0 g |
| CaCl <sub>2</sub> . 2H <sub>2</sub> O ..... | 0.33 g  |
| MgSO <sub>4</sub> . 7H <sub>2</sub> O ..... | 4.0 g   |
| Resazurin.....                              | 2.0 mg  |
| Wolfe's Vitamin Solution (see below)....    | 10.0 ml |
| Wolfe's Mineral Solution (see below)....    | 10.0 ml |
| Yeast extract.....                          | 0.5 g   |
| Sucrose.....                                | 5.0     |
| NaHCO <sub>3</sub> .....                    | 2.0 g   |
| Na <sub>2</sub> S . 9H <sub>2</sub> O ..... | 0.5 g   |
| Distilled water.....                        | 1.0 L   |

Boil medium minus NaHCO<sub>3</sub> and Na<sub>2</sub>S . 9H<sub>2</sub>O and cool under 80% N<sub>2</sub>, 10% CO<sub>2</sub>, 10% H<sub>2</sub> gas mixture. Add NaHCO<sub>3</sub> then Na<sub>2</sub>S . 9H<sub>2</sub>O. Adjust for final pH 7.0. Tube anaerobically under same gas phase. Autoclave at 121C for 15 minutes.

*Wolfe's Vitamin Solution:*

|                                     |         |
|-------------------------------------|---------|
| 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 <sub>4</sub> . 7H <sub>2</sub> O .....                  | 3.0 g  |
| MnSO <sub>4</sub> . H <sub>2</sub> O .....                   | 0.5 g  |
| NaCl.....  | 1.0 g  |
| FeSO <sub>4</sub> . 7H <sub>2</sub> O .....                  | 0.1 g  |
| CoCl <sub>2</sub> . 6H <sub>2</sub> O .....                  | 0.1 g  |
| CaCl <sub>2</sub> .....                                      | 0.1 g  |
| ZnSO <sub>4</sub> . 7H <sub>2</sub> O .....                  | 0.1 g  |
| CuSO <sub>4</sub> . 5H <sub>2</sub> O .....                  | 0.01 g |
| AlK(SO <sub>4</sub> ) <sub>2</sub> . 12H <sub>2</sub> O..... | 0.01 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  |

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.