

**ATCC medium: 2006 *Desulfobacterium anilini* medium (DSM 193 with phenol)**

*Solution A:*

|   |          |
|---|----------|
| Na <sub>2</sub> SO <sub>4</sub> .....       | 3.0 g    |
| KH <sub>2</sub> PO <sub>4</sub> .....       | 0.2 g    |
| NH <sub>4</sub> Cl .....                    | 0.3 g    |
| NaCl.....                                   | 7.0 g    |
| MgCl <sub>2</sub> . 6H <sub>2</sub> O ..... | 1.3 g    |
| KCl.....                                    | 0.5 g    |
| CaCl <sub>2</sub> . 2H <sub>2</sub> O ..... | 0.15 g   |
| Resazurin.....                              | 1.0 mg   |
| Distilled water.....                        | 870.0 ml |

Heat to boiling and cool to room temperature under 80% N<sub>2</sub>, 20% CO<sub>2</sub> until pH drops below 6. Autoclave under the same gas phase at 121C for 15 minutes.

*Solution B:*

|   |        |
|---|--------|
| Trace Elements Solution SL-10 (see below) ..... | 1.0 ml |
|---|--------|

Autoclave under N<sub>2</sub> at 121C for 15 minutes.

*Solution C:*

|                          |          |
|--------------------------|----------|
| NaHCO <sub>3</sub> ..... | 5.0 g    |
| Distilled water.....     | 100.0 ml |

Filter-sterilize and flush with 80% N<sub>2</sub>, 20% CO<sub>2</sub>.

*Solution D:*

|                      |         |
|----------------------|---------|
| Phenol.....          | 94.0 mg |
| Distilled water..... | 10.0 ml |

Freshly prepare, filter-sterilize, and equilibrate under N<sub>2</sub>.

*Solution E:*

|  |         |
|--|---------|
| Wolfe's Vitamin Solution (see below) ..... | 10.0 ml |
|--|---------|

Autoclave under N<sub>2</sub> at 121C for 15 minutes.

*Solution F:*

|   |        |
|---|--------|
| NaOH.....   | 0.5 g  |
| Na <sub>2</sub> SeO <sub>3</sub> . 5H <sub>2</sub> O..... | 3.0 mg |
| Na <sub>2</sub> WO <sub>4</sub> . 2H <sub>2</sub> O.....  | 4.0 mg |
| Distilled water.....                                      | 1.0 L  |

Autoclave under N<sub>2</sub> at 121C for 15 minutes. Use 1.0 ml/L medium.

*Solution G:*

|   |         |
|---|---------|
| Na <sub>2</sub> S . 9H <sub>2</sub> O ..... | 0.4 g   |
| Distilled water.....                        | 10.0 ml |

Autoclave under N<sub>2</sub> at 121C for 15 minutes.

Aseptically add solutions B through G to sterile, cooled solution A in the sequence indicated. Dispense completed medium anaerobically under a gas phase of 80% N<sub>2</sub>, 20% CO<sub>2</sub> into appropriate vessels. Final pH 7.1-7.4.

*Trace Elements Solution SL-10:*

|   |          |
|---|----------|
| HCl (25%) .....   | 10.0 ml  |
| FeCl <sub>2</sub> . 4H <sub>2</sub> O .....               | 1.5 g    |
| ZnCl <sub>2</sub> .....                                   | 70.0 mg  |
| MnCl <sub>2</sub> . 4H <sub>2</sub> O .....               | 100.0 mg |
| H <sub>3</sub> BO <sub>3</sub> .....                      | 6.0 mg   |
| CoCl <sub>2</sub> . 6H <sub>2</sub> O .....               | 190.0 mg |
| CuCl <sub>2</sub> . 2H <sub>2</sub> O .....               | 2.0 mg   |
| NiCl <sub>2</sub> . 6H <sub>2</sub> O .....               | 24.0 mg  |
| Na <sub>2</sub> MoO <sub>4</sub> . 2H <sub>2</sub> O..... | 36.0 mg  |
| Distilled water.....                                      | 990.0 ml |

Dissolve FeCl<sub>2</sub> in the HCl, dilute with water, add and dissolve the other salts; adjust pH to 6.0 with NaOH, and fill to 1.0 L with distilled water.

*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   |