

**385: DESULFOCASTAENA MEDIUM**

|                   |        |    |
|-------------------|--------|----|
| <b>Solution A</b> | 952.00 | ml |
| <b>Solution B</b> | 20.00  | ml |
| <b>Solution C</b> | 10.00  | ml |
| <b>Solution D</b> | 10.00  | ml |
| <b>Solution E</b> | 1.00   | ml |
| <b>Solution F</b> | 10.00  | ml |

1. Solution A is sparged with 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas atmosphere. Solutions C and F are autoclaved under 100% N<sub>2</sub> gas. Solutions D and E are prepared under 100% N<sub>2</sub> gas and sterilized by filtration. The pyrocatechol stock solution has to be prepared always freshly prior to use. To complete the medium appropriate amounts of the solutions B to F are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be at 6.9 - 7.1.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N<sub>2</sub> and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 10 - 20% inoculum.

**Solution A**

|  |        |    |
|--|--------|----|
| Na <sub>2</sub> SO <sub>4</sub>        | 3.00   | g  |
| KH <sub>2</sub> PO <sub>4</sub>        | 0.20   | g  |
| NH <sub>4</sub> Cl                     | 0.30   | g  |
| NaCl                                   | 7.00   | g  |
| MgCl <sub>2</sub> x 6 H <sub>2</sub> O | 1.30   | g  |
| KCl                                    | 0.50   | g  |
| CaCl <sub>2</sub> x 2 H <sub>2</sub> O | 0.15   | g  |
| <b>Trace element solution SL-10</b>    | 1.00   | ml |
| <b>Selenite-tungstate solution</b>     | 1.00   | ml |
| Sodium resazurin (0.1% w/v)            | 0.50   | ml |
| Distilled water                        | 950.00 | ml |

**Solution B**

|                                 |       |    |
|---------------------------------|-------|----|
| Na <sub>2</sub> CO <sub>3</sub> | 1.00  | g  |
| Distilled water                 | 20.00 | ml |

**Solution C**

|             |      |   |
|-------------|------|---|
| Na-benzoate | 0.40 | g |
|-------------|------|---|

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|                 |       |    |
|-----------------|-------|----|
| Distilled water | 10.00 | ml |
|-----------------|-------|----|

### Solution D

|                 |       |    |
|-----------------|-------|----|
| Pyrocatechol    | 0.06  | g  |
| HCl (1 N)       | 0.06  | ml |
| Distilled water | 10.00 | ml |

### Solution E

|                                |      |    |
|--------------------------------|------|----|
| Wolin's vitamin solution (10x) | 1.00 | ml |
|--------------------------------|------|----|

### Solution F

|  |       |    |
|--|-------|----|
| Na <sub>2</sub> S x 9 H <sub>2</sub> O | 0.40  | g  |
| Distilled water                        | 10.00 | ml |

### Selenite-tungstate solution (from medium 385)

|   |         |    |
|---|---------|----|
| NaOH  | 0.50    | g  |
| Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O | 3.00    | mg |
| Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O  | 4.00    | mg |
| Distilled water                                       | 1000.00 | ml |

### Trace element solution SL-10 (from medium 320)

|   |        |    |
|---|--------|----|
| HCl (25%)   | 10.00  | ml |
| FeCl <sub>2</sub> x 4 H <sub>2</sub> O                | 1.50   | g  |
| ZnCl <sub>2</sub>                                     | 70.00  | mg |
| MnCl <sub>2</sub> x 4 H <sub>2</sub> O                | 100.00 | mg |
| H <sub>3</sub> BO <sub>3</sub>                        | 6.00   | mg |
| CoCl <sub>2</sub> x 6 H <sub>2</sub> O                | 190.00 | mg |
| CuCl <sub>2</sub> x 2 H <sub>2</sub> O                | 2.00   | mg |
| NiCl <sub>2</sub> x 6 H <sub>2</sub> O                | 24.00  | mg |
| Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O | 36.00  | mg |
| Distilled water                                       | 990.00 | ml |

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

### Wolin's vitamin solution (10x) (from medium 120)

|                          |        |    |
|--------------------------|--------|----|
| Biotin                   | 20.00  | mg |
| Folic acid               | 20.00  | mg |
| Pyridoxine hydrochloride | 100.00 | mg |
| Thiamine HCl             | 50.00  | mg |

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|                            |         |    |
|----------------------------|---------|----|
| Riboflavin                 | 50.00   | mg |
| Nicotinic acid             | 50.00   | mg |
| Calcium D-(+)-pantothenate | 50.00   | mg |
| Vitamin B <sub>12</sub>    | 1.00    | mg |
| p-Aminobenzoic acid        | 50.00   | mg |
| (DL)-alpha-Lipoic acid     | 50.00   | mg |
| Distilled water            | 1000.00 | ml |