

496: PSEUDODESULFOVIBRIO MEDIUM

| | | |
|-------------------|--------|----|
| Solution A | 962.00 | ml |
| Solution B | 20.00 | ml |
| Solution C | 10.00 | ml |
| Solution D | 1.00 | ml |
| Solution E | 10.00 | ml |

1. Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture to reach a pH below 6 (at least 30 min), then dispensed under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and E are autoclaved under 100% N₂ gas. Solution D is filter-sterilized and gassed with 100% N₂ gas. To complete the medium appropriate amounts of solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 6.8 – 7.2.

2. Note: Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution freshly prepared under N₂ and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

Solution A

| | | |
|--|--------|----|
| Na ₂ SO ₄ | 3.00 | g |
| KH ₂ PO ₄ | 0.20 | g |
| NH ₄ Cl | 0.30 | g |
| NaCl | 70.00 | g |
| MgCl ₂ x 6 H ₂ O | 3.00 | g |
| KCl | 0.50 | g |
| CaCl ₂ x 2 H ₂ O | 0.15 | g |
| Trace element solution SL-10 | 1.00 | ml |
| Selenite-tungstate solution | 1.00 | ml |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| Distilled water | 960.00 | ml |

Solution B

| | | |
|---------------------------------|-------|----|
| Na ₂ CO ₃ | 1.00 | g |
| Distilled water | 20.00 | ml |

Solution C

| | | |
|-----------------|-------|----|
| Na-L-lactate | 2.50 | g |
| Distilled water | 10.00 | ml |

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Solution D

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

Solution E

| | | |
|--|-------|----|
| Na ₂ S x 9 H ₂ O | 0.40 | g |
| Distilled water | 10.00 | ml |

Trace element solution SL-10 (from medium 320)

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

First dissolve FeCl₂ in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

Selenite-tungstate solution (from medium 385)

| | | |
|---|---------|----|
| NaOH | 0.50 | g |
| Na ₂ SeO ₃ x 5 H ₂ O | 3.00 | mg |
| Na ₂ WO ₄ x 2 H ₂ O | 4.00 | mg |
| Distilled water | 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 |
| Riboflavin | 50.00 | mg |
| Nicotinic acid | 50.00 | mg |
| Calcium D-(+)-pantothenate | 50.00 | mg |
| Vitamin B ₁₂ | 1.00 | mg |
| p-Aminobenzoic acid | 50.00 | mg |
| (DL)-alpha-Lipoic acid | 50.00 | mg |
| Distilled water | 1000.00 | ml |