

149: MEGADESULFOVIBRIO MEDIUM

| | | |
|-------------------|--------|----|
| Solution A | 970.00 | ml |
| Solution B | 20.00 | ml |
| Solution C | 1.00 | ml |
| Solution D | 10.00 | ml |

1. Sparge solution A with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic, then dispense under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. Solution C is prepared under 100% N₂ gas atmosphere and sterilized by filtration. Solution D is autoclaved separately under 100% N₂ gas. To complete the medium appropriate amounts of solutions B to D are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 7.0 - 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% inoculum.

Solution A

| | | |
|--|--------|----|
| KH ₂ PO ₄ | 1.00 | g |
| NH ₄ Cl | 0.50 | g |
| MgSO ₄ x 7 H ₂ O | 0.40 | g |
| Na ₂ SO ₄ | 2.00 | g |
| CaCl ₂ x 2 H ₂ O | 0.10 | g |
| Trace element solution SL-6 | 1.00 | ml |
| H ₂ SO ₄ (2 N) | 1.00 | ml |
| Na-L-lactate | 2.00 | g |
| Sodium resazurin (0.1% w/v) | 0.50 | ml |
| Distilled water | 970.00 | ml |

Solution B

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

Solution C

| | | |
|--------------------------------|------|----|
| Seven vitamins solution | 1.00 | ml |
|--------------------------------|------|----|

Solution D

| | | |
|--|------|---|
| Na ₂ S x 9 H ₂ O | 0.30 | g |
|--|------|---|

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

Trace element solution SL-6 (from medium 27)

| | | |
|---|---------|----|
| ZnSO ₄ x 7 H ₂ O | 0.10 | g |
| MnCl ₂ x 4 H ₂ O | 0.03 | g |
| H ₃ BO ₃ | 0.30 | g |
| CoCl ₂ x 6 H ₂ O | 0.20 | g |
| CuCl ₂ x 2 H ₂ O | 0.01 | g |
| NiCl ₂ x 6 H ₂ O | 0.02 | g |
| Na ₂ MoO ₄ x 2 H ₂ O | 0.03 | g |
| Distilled water | 1000.00 | ml |

Seven vitamins solution (from medium 503)

| | | |
|-----------------------------------|---------|----|
| Vitamin B ₁₂ | 100.00 | mg |
| p-Aminobenzoic acid | 80.00 | mg |
| D-(+)-biotin | 20.00 | mg |
| Nicotinic acid | 200.00 | mg |
| Calcium pantothenate | 100.00 | mg |
| Pyridoxine hydrochloride | 300.00 | mg |
| Thiamine-HCl x 2 H ₂ O | 200.00 | mg |
| Distilled water | 1000.00 | ml |