

833. DEHALOSPIRILLUM MEDIUM

Solution A:

Na ₂ SO ₄	0.70	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.25	g
NaCl	0.25	g
MgCl ₂ x 6 H ₂ O	0.40	g
KCl	0.50	g
CaCl ₂ x 2 H ₂ O	0.15	g
Yeast extract	2.00	g
Trace element solution SL-10 (see medium 320)	1.00	ml
Selenite-tungstate solution (see medium 385)	1.00	ml
Na-resazurin solution (0.1% w/v)	0.50	ml
Distilled water	875.00	ml

Solution B:

Potassium phosphate buffer (0.1 M, pH 7.5)	10.00	ml
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Solution C:

Vitamin solution (see medium 141)	9.00	ml
Vitamin solution (see medium 503)	1.00	ml

Solution D:

NaHCO ₃	3.50	g
Distilled water	45.00	ml

Solution E:

Na-pyruvate	4.50	g
Distilled water	20.00	ml

Solution F:

Na ₂ -fumarate	6.40	g
Distilled water	40.00	ml

Solution G:

FeSO ₄ x 7 H ₂ O	25.00	mg
0.1 N H ₂ SO ₄	1.00	ml

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Solution H:

L-Cysteine-HCl x H ₂ O	50.00 mg
Distilled water	1.00 ml

Sparge *solution A* with 100% N₂ gas for 30 – 45 min to make it anoxic, then distribute under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. *Solutions B* and *H* are autoclaved separately under 100% N₂ gas. *Solutions C, E, F* and *G* are prepared under 100% N₂ gas and sterilized by filtration. *Solution D* is autoclaved under 80% N₂ and 20% CO₂ gas atmosphere. To complete the medium appropriate amounts of *solutions B* to *H* are added to the sterile *solution A* in the sequence as indicated. Adjust pH of complete medium to 7.3 - 7.6, if necessary.

Note: For resuscitation of freeze-dried strains from ampoule, the medium must be totally reduced, i.e. resazurin has to be colorless. Addition of 10 - 20 mg sodium dithionite per liter (e.g. from 5% (w/v) solution, freshly prepared under N₂ and filter-sterilized) may help to reduce the medium.