

739. DESULFONEMA ISHIMOTOI MEDIUM**Solution A:**

NaCl	25.00	g
MgCl ₂ x 6 H ₂ O	5.60	g
MgSO ₄ x 7 H ₂ O	6.80	g
CaCl ₂ x 2 H ₂ O	1.40	g
KCl	0.72	g
KH ₂ PO ₄	0.14	g
NH ₄ Cl	0.25	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	1000.00	ml

Solution B:

Na-acetate x 3 H ₂ O	2.50	g
Na ₂ -succinate	0.10	g
Isobutyric acid	0.19	ml
Distilled water	10.00	ml

Adjust to pH 7.0 with 1 N NaOH.

Solution C:

Vitamin solution (see medium 141)	10.00	ml
-----------------------------------	-------	----

Solution D:

KAl(SO ₄) ₂ x 12 H ₂ O	0.48	g
Distilled water	10.00	ml

Solution E:

Na ₂ CO ₃	1.00	g
Distilled water	20.00	ml

Solution F:

Na ₂ S x 9 H ₂ O	0.30	g
Distilled water	10.00	ml

Sparge *solution A* with 80% N₂ and 20% CO₂ gas mixture for 30 – 45 min to make it anoxic and reach a pH of around 6, then distribute under the same gas atmosphere into anoxic Hungate-type tubes and autoclave.

Continued on next page

Solutions B and C are prepared under 100% N₂ gas atmosphere and sterilized by filtration. *Solutions D and F* are autoclaved separately under 100% N₂ gas. *Solution E* is prepared under 80% N₂ and 20% CO₂ gas atmosphere. To complete the medium appropriate amounts of *solutions B to F* are added to the sterile *solution A* in the sequence as indicated. Final pH of the medium should be at 7.0. After combining solutions the medium should equilibrate overnight and a white precipitate should be apparent.

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