

202: DESULFONEMA MAGNUM MEDIUM

Solution A	952.00	ml
Solution B	10.00	ml
Solution C	1.00	ml
Solution D	10.00	ml
Solution E	20.00	ml
Solution F	10.00	ml

1. Solution A is sparged with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to reach a pH of around 6, then distributed under the same gas atmosphere into anoxic Hungate-type tubes and autoclaved. Solutions B, D, and F are autoclaved separately under 100% N₂ gas. Solution E is autoclaved under 80% N₂ and 20% CO₂ gas mixture. Solution C is prepared under 100% N₂ gas and sterilized by filtration. 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 7.0. After completion the medium should equilibrate overnight and a white precipitate should be apparent.

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) just before inoculation may stimulate growth at the beginning. For transfers use 5 - 10% inoculum.

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	1.00	ml
Selenite-tungstate solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Na-benzoate	0.60	g
Distilled water	10.00	ml

Solution C

Wolin's vitamin solution (10x)	1.00	ml
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Solution D

$\text{AlK}(\text{SO}_4)_2 \times 12 \text{H}_2\text{O}$	0.48	g
Distilled water	10.00	ml

Solution E

Na_2CO_3	1.00	g
Distilled water	20.00	ml

Solution F

$\text{Na}_2\text{S} \times 9 \text{H}_2\text{O}$	0.30	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$\text{FeCl}_2 \times 4 \text{H}_2\text{O}$	1.50	g
ZnCl_2	70.00	mg
$\text{MnCl}_2 \times 4 \text{H}_2\text{O}$	100.00	mg
H_3BO_3	6.00	mg
$\text{CoCl}_2 \times 6 \text{H}_2\text{O}$	190.00	mg
$\text{CuCl}_2 \times 2 \text{H}_2\text{O}$	2.00	mg
$\text{NiCl}_2 \times 6 \text{H}_2\text{O}$	24.00	mg
$\text{Na}_2\text{MoO}_4 \times 2 \text{H}_2\text{O}$	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl_2 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
$\text{Na}_2\text{SeO}_3 \times 5 \text{H}_2\text{O}$	3.00	mg
$\text{Na}_2\text{WO}_4 \times 2 \text{H}_2\text{O}$	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



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p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml