

383: DESULFOBACTERIUM MEDIUM

This recipe contains strain-specific modifications for *Desulfosarcina ovata* DSM 13228 *

Final pH: 7.0 - 7.2

Final volume: 1003 ml

Solution A	952.00	ml
Solution B	20.00	ml
Solution C	20.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Seven vitamins solution	1.00	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 distributed in anoxic cultivation vials and autoclaved under the same gas atmosphere. Solution B is autoclaved separately under 80% N₂ and 20% CO₂ gas atmosphere. Solutions C and D are prepared under 100% N₂ gas and filter-sterilized. Solution E is autoclaved under 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 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. Incubate all strains in the dark.

* Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503). Replace pyruvate with 0.40 g/l benzoate and 0.10 g/l yeast extract sterilized separately by filtration and add to the autoclaved medium from anoxic stock solutions.

Solution A

Na ₂ SO ₄	3.00	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
NaCl	21.00	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	950.00	ml

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Solution B

Na ₂ CO ₃	1.00	g
Distilled water	20.00	ml

Solution C

Na-pyruvate	2.50	g
Na-benzoate	0.40	g
Yeast extract	0.10	g
Distilled water	20.00	ml

Solution D

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

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

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.

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
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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

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