

194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

Solution A	952.00	ml
Solution B	30.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	10.00	ml

1. Solution A is sparged with 80% N_2 and 20% CO_2 gas mixture to reach a pH below 6 (at least 30 min), then distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and E are autoclaved under 100% N_2 gas. Solution D is prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solutions B to E are added to the sterile, cooled solution A in appropriate amounts in the sequence as indicated. Final pH of the medium should be 7.1 - 7.4.

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% (v/v) inoculum.

For <u>DSM 2055</u>: Na-propionate is replaced with 0.70 g/l Na-butyrate, 0.30 g/l Na-caproate and 0.15 g/l Na-octanoate added after autoclaving from sterile anoxic stock solutions prepared under N_2 . Adjust final pH of medium to 7.7.

For <u>DSM 3852</u>: Na-propionate is replaced with 0.70 g/l ethanol absolute added after autoclaving from a sterile anoxic stock solution prepared under N_2 . In addition, the medium is supplemented with 0.10 g/l each of yeast extract and casamino acids.

For <u>DSM 5092</u>: Na-propionate is replaced with 0.90 g/l putrescine added after autoclaving from a sterile anoxic stock solution prepared under N_2 .

For <u>DSM 5193</u>: Supplement medium with 0.50 g/l yeast extract and replace Na-propionate with 0.90 g/l Na-methoxyacetate added after autoclaving from a sterile anoxic stock solution prepared under N_2 .

For <u>DSM 5433</u>: Na-propionate is replaced with 1.50 g/l 1,2-propanediol added after autoclaving from a sterile anoxic stock solution prepared under N_2 .

For <u>DSM 5651</u>: Na-propionate is replaced with 1.50 g/l of Na-(D/L)-3-hydroxybutyrate as substrate.

For <u>DSM 6283</u>, <u>DSM 12907</u>: Na-propionate is replaced with 2.50 g/l Na-(DL)-lactate and 1.00 g/l yeast extract added after autoclaving from sterile anoxic stock solutions prepared under N_2 . Final pH of the medium should be 6.8 - 7.0.

For <u>DSM 7474</u>, <u>DSM 7475</u>, <u>DSM 7476</u>: Na-propionate is replaced by 0.40 g/l 3,4,5-trimethoxybenzoic acid added after autoclaving from a sterile anoxic stock solution (2% w/v) prepared under N₂ and neutralized with NaOH. Stock solutions of 3,4,5-trimethoxybenzoate should be prepared freshly and sterilized by filtration.

Microorganisms

194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)



Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added from an anoxic stock solution sterilized by filtration.

For <u>DSM 10291</u>, <u>DSM 24454</u>: Na-propionate is replaced with 2.50 g/l Na-L-lactate added after autoclaving from a sterile anoxic stock solution prepared under N₂. Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added from an anoxic stock solution sterilized by filtration.

For <u>DSM 12016</u>: Na-propionate is replaced with 0.10 ml/l propanol added after autoclaving from a sterile anoxic stock solution prepared under N₂. Supplement medium with 1.00 g/l yeast extract. When growth has started, feed again same amount of propanol.

For <u>DSM 13527</u>, <u>DSM 21556</u>, <u>DSM 24590</u>: Na-propionate is replaced with 1.00 g/l Na -butyrate added after autoclaving from a sterile anoxic stock solution prepared under N_2 .

For <u>DSM 14880</u>: Na-propionate is replaced with 0.50 g/l yeast extract and 2.20 g/l sodium pyruvate added after autoclaving from anoxic stock solutions sterilized by filtration.

For <u>DSM 28570</u>: Na-propionate is replaced with 0.15 g/l Na-benzoate added after autoclaving from a sterile, anoxic stock solution prepared under N_2 .

For <u>DSM 102358</u>, <u>DSM 102359</u>, <u>DSM 102360</u>: Na-propionate is replaced with 1.50 g/ Na -benzoate and 1.00 g/l Na-acetate as substrates. Reduce amount of sulfide to 0.10 g/l. Dithionite is used to reduce medium completely.

For <u>DSM 105758</u>: Na-propionate is replaced with 0.70 g/l Na-butyrate, 0.30 g/l Na-caproate and 0.15 g/l Na-octanoate added after autoclaving from sterile anoxic stock solutions prepared under N_2 . Adjust final pH of medium to 9.0.

For <u>DSM 107641</u>: Na-propionate is replaced with 1.50 g/ 1,2-propanediol added after autoclaving from a sterile, anoxic stock solution prepared under N_2 .

Solution A		
Na ₂ SO ₄	3.00	g
KH ₂ PO ₄	0.20	g
NH ₄ Cl	0.30	g
NaCl	1.00	g
$MgCl_2 \times 6 H_2O$	0.40	g
KCI	0.50	g
$CaCl_2 \times 2 H_2O$	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

Solution B

 Na_2CO_3

1.50 g

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Microorganisms



194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

Distilled water	30.00	ml
Solution C Na-propionate Distilled water	1.50 10.00	g ml
Solution D Wolin's vitamin solution (10x)	1.00	ml
Solution E Na ₂ S x 9 H ₂ O Distilled water	0.40 10.00	g ml
Trace element solution SL-10 (from medium	n 320)	
	10.00	mi
ZnCl ₂	70.00	ma
$MnCl_2 \times 4 H_2O$	100.00	mg
H ₃ BO ₃	6.00	mg
$CoCl_2 \times 6 H_2O$	190.00	mg
$CuCl_2 \times 2 H_2O$	2.00	mg
NICI ₂ x 6 H_2O	24.00	mg
Distilled water	990.00	mg 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 0.5) g
$Na_2SeO_3 \times 5 H_2O$ 3.0) mg
$Na_2WO_4 \times 2 H_2O$ 4.0) mg
Distilled water 1000.0) ml

Wolin's vitamin solution (10x) (from med	lium 120)	
Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg

Microorganisms



194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

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