194: DESULFOBULBUS SP. MEDIUM (FRESHWATER)

This recipe contains strain-specific modifications for *Desulfofundulus sp.* DSM 7475 *

Final pH: 7.1 - 7.4
Final volume: 1003 ml

Solution A	952.00	ml
Solution B	30.00	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% 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 N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% (v/v) inoculum.

* 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 N_2 and neutralized with NaOH. Stock solutions of 3,4,5-trimethoxybenzoate should be prepared freshly and sterilized by filtration. Supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added from an anoxic stock solution sterilized by filtration.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	1.00	g
$MgCl_2 \times 6\ H_2O$	0.40	g
KCl	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
Distilled water	30.00	ml

Solution C

~~Na propionate~~	~~1.50~~	~~g~~
3,4,5-Trimethoxybenzoic acid	0.40	g
Distilled water	20.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

$Na_2S \times 9\ H_2O$	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2 \times 4\ H_2O$	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2 \times 4\ H_2O$	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2 \times 6\ H_2O$	190.00	mg
$CuCl_2 \times 2\ H_2O$	2.00	mg
$NiCl_2 \times 6\ H_2O$	24.00	mg
$Na_2MoO_4 \times 2\ H_2O$	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
$Na_2SeO_3 \times 5\ H_2O$	3.00	mg
$Na_2WO_4 \times 2\ H_2O$	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
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_2O	200.00	mg
Distilled water	1000.00	ml