383: DESULFOBACTERIUM MEDIUM

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

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 in anoxic cultivation vials and autoclaved under the same gas atmosphere. Solution B is autoclaved separately under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas and filter-sterilized. Solution E is autoclaved under 100% N_2 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 N_2 and filter-sterilized) may stimulate growth of some strains at the beginning. For transfers use 5 - 10% inoculum. Incubate all strains in the dark.

For DSM 2056: Replace pyruvate with 0.70 g/l Na-butyrate, 0.30 g/l Na-caproate, and 0.15 g/l sodium octanoate.

For DSM 3383: Replace pyruvate with indole as substrate. Dissolve 0.30 g of indole in 90 ml water by heating and shaking in a closed bottle under 100% N_2 gas atmosphere, autoclave, then add 7.00 ml of a sterile anoxic stock solution of NaCl (30% w/v) and 0.70 ml of a sterile anoxic stock solution of $MgCl_2$ x 6 H_2O (40% w/v). Store the indole-salt solution in the dark. Reheat and shake before use. Add to sterile medium 30.00 ml/l of the indole-salt solution in the beginning, and 2 x 30.00 ml/l during growth.

For DSM 3384: The vitamin solution of medium 120 is replaced with 1.00 ml/l of seven vitamins solution of medium 503. Replace pyruvate with 0.40 g/l Na-benzoate and add 10.00 ml/l clarified rumen fluid (see medium 1310) as growth supplement. Initiation of growth requires addition of freshly prepared sodium dithionite.

For DSM 4661: Replace pyurvate with resorcinol. During growth the culture is fed once with the same amount of resorcinol.

For DSM 5091, DSM 9788: Replace pyruvate with 2.00 g/l malonic acid.

For DSM 7044, DSM 7120, DSM 7467, DSM 12567, DSM 13228, DSM 23484: 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.

For DSM 8540: Replace pyruvate with 0.30 g/l p-hydroxybenzoate.

For DSM 9705: Replace pyruvate with 1.00 g/l Na-glycolate sterilized by filtration.

For DSM 12861, DSM 12883: Replace pyruvate with 1.00 g/l valeric acid.

For DSM 12888: Replace pyruvate with 1.10 g/l Na-butyrate and 1.10 g/l caproic acid.

For DSM 13036: Replace pyruvate with 1.00 g/l betaine.

For DSM 14454: Naphthalene dissolved in heptamethylnonane can be used as substrate instead of pyruvate.

For DSM 15576, DSM 16219: Replace pyruvate with 1.00 g/l Na-caprylate and supplement medium with 1.00 ml/l seven vitamins solution (see medium 503) added to the autoclaved medium from an anoxic stock solution sterilized by filtration.

For DSM 17291: Replace pyruvate with 3.00 g/l Casamino acids.

For DSM 17477: Replace pyruvate with 1.90 g/l sodium glutamate monohydrate and 1.00 g/l yeast extract.

For DSM 21856: Replace pyruvate with 0.90 ml/l 1-butanol.

For DSM 28890: Replace pyruvate with 0.50 g/l yeast extract and 0.68 g/l sodium formate.

For DSM 100305, DSM 105015: Change amount of pyruvate to 2.20 g/l and add 0.10 g/l yeast extract.

Solution A

Na_2SO_4	3.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.30	g
NaCl	21.00	g
$MgCl_2$ x 6 H_2O	3.00	g
KCl	0.50	g
$CaCl_2$ x 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	1.00	g
Distilled water	20.00	ml

Solution C

Na-pyruvate	2.50	g
Distilled water	20.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	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_2O	1.50	g
$ZnCl_2$	70.00	mg
$MnCl_2$ x 4 H_2O	100.00	mg
H_3BO_3	6.00	mg
$CoCl_2$ x 6 H_2O	190.00	mg
$CuCl_2$ x 2 H_2O	2.00	mg
$NiCl_2$ x 6 H_2O	24.00	mg
Na_2MoO_4 x 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.

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