861: DESULFOFRIGUS MEDIUM

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
Solution F	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 - 45 min), then dispensed under the same gas atmosphere into serum vials (use at least bottles of 50 ml volume filled with 30 ml medium) and autoclaved. Solutions B and F are prepared under 80% N_2 and 20% CO_2 gas atmosphere and sterilized. Sterile solutions C, D and E are prepared under 100% N_2 gas. Solutions D and F are 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 - 7.2.

2. Note: For transfer of cultures use at least 10% (v/v) inoculum.

For DSM 12341: Replace Na-DL-lactate with 1.50 g/l Na-acetate.

For DSM 12344: Replace Na-DL-lactate with 1.50 g/l Na-propionate.

Solution A

NaCl	20.00	g
Na_2SO_4	4.00	g
KH_2PO_4	0.20	g
NH_4Cl	0.25	g
$MgCl_2$ x 6 H_2O	3.00	g
$CaCl_2$ x 2 H_2O	0.15	g
KBr	0.09	g
KCl	0.50	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-DL-lactate	2.50	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

$Na_2S \times 9\ H_2O$	0.30	g
Distilled water	10.00	ml

Solution F

Na-dithionite solution (5% w/v)	1.00	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

Na-dithionite solution (5% w/v) (from medium 829)

$NaHCO_3$	50.00	g
$Na_2S_2O_4$	50.00	g
Distilled water	1000.00	ml

Dissolve $NaHCO_3$ in water and make the solution anoxic by sparging with 80% N_2 and 20% CO_2 gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.