875: DESULFACINUM HYDROTHERMALE MEDIUM

Solution A	950.00	ml
Solution B	1.00	ml
Solution C	30.00	ml
Solution D	1.00	ml
Solution E	10.00	ml
Solution F	10.00	ml

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 distributed under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclaved. Solutions B, E and F are autoclaved separately under 100% N_2 gas atmosphere. Solution C is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solution D is prepared under 100% N_2 gas atmosphere and 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 - 7.3.

Solution A

NaCl	26.00	g
$MgCl_2$ x 6 H_2O	5.60	g
$CaCl_2$ x 2 H_2O	1.40	g
$MgSO_4$ x 7 H_2O	6.80	g
NH_4Cl	0.25	g
KH_2PO_4	0.20	g
KCl	0.72	g
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	950.00	ml

Solution B

Trace element solution SL-10	1.00	ml

Solution C

Na_2CO_3	1.50	g
Distilled water	30.00	ml

Solution D

Seven vitamins solution	1.00	ml

Solution E

Na-DL-lactate	2.50	g
Distilled water	10.00	ml

Solution F

Na_2S x 9 H_2O	0.40	g
Distilled water	10.00	ml

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
$FeCl_2$ 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.

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