1665: DESULFAMPLUS MEDIUM

NaCl	20.00	g
$MgCl_2$ x 6 H_2O	6.00	g
Na_2SO_4	3.00	g
$CaCl_2$ x 2 H_2O	0.20	g
KCl	0.50	g
NH_4Cl	0.30	g
Wolfe's mineral elixir	2.00	ml
HEPES (SIGMA)	2.40	g
Na_2-fumarate	1.00	g
Yeast extract (OXOID)	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
K-phosphate buffer (0.5 M, pH 7.0)	2.00	ml
$NaHCO_3$	1.00	g
$FeCl_2$ x 4 H_2O (0.2% w/v in 0.02 N HCl)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients (except phosphate buffer, bicarbonate, ferrous chloride, vitamins and cysteine), adjust pH to 7.2 with NaOH and sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After autoclaving add phosphate buffer, ferrous chloride, vitamins and cysteine from sterile anoxic stock solutions prepared under 100% N_2 gas atmosphere and bicarbonate from a sterile stock solution prepared under 80% N_2 and 20% CO_2 gas mixture. Vitamins should be sterilized by filtration. Adjust pH of the complete medium to 7.2 - 7.5 , if necessary.

Wolfe's mineral elixir (from medium 792)

$MgSO_4$ x 7 H_2O	30.00	g
$MnSO_4$ x H_2O	5.00	g
NaCl	10.00	g
$FeSO_4$ x 7 H_2O	1.00	g
$CoCl_2$ x 6 H_2O	1.80	g
$CaCl_2$ x 2 H_2O	1.00	g
$ZnSO_4$ x 7 H_2O	1.80	g
$CuSO_4$ x 5 H_2O	0.10	g
$AlK(SO_4)_2$ x 12 H_2O	0.18	g
H_3BO_3	0.10	g
Na_2MoO_4 x 2 H_2O	0.10	g
$(NH_4)_2Ni(SO_4)_2$ x 6 H_2O	2.80	g
Na_2WO_4 x 2 H_2O	0.10	g

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Na_2SeO_4	0.10	g
Distilled water	1000.00	ml

First adjust pH to 1.0 with diluted H_2SO_4, then add and dissolve the salts.

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

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