525a: METHANOHALOPHILUS (MG+NaCl) MEDIUM

KCl	0.34	g
$MgCl_2$ x 6 H_2O	2.75	g
$MgSO_4$ x 7 H_2O	3.45	g
NH_4Cl	0.25	g
K_2HPO_4 x 3 H_2O	0.14	g
$CaCl_2$ x 2 H_2O	0.14	g
NaCl	150.00	g
Modified Wolin's mineral solution	10.00	ml
Na-acetate	1.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
$NaHCO_3$	4.00	g
Na_2CO_3	0.50	g
Trimethylamine-HCl	5.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H_2O	0.50	g
Na_2S x 9 H_2O	0.50	g
Distilled water	1000.00	ml

Dissolve ingredients except carbonates, trimethylamine, vitamins, cysteine and sulfide, then sparge medium with 80% N_2 and 20% CO_2 gas mixture for 30 - 45 min to make it anoxic. Dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Complete medium by adding sterile anoxic stock solutions of trimethylamine, vitamins (sterilized by filtration), cysteine and sulfide prepared under 100% N_2 gas atmosphere and bicarbonate and carbonate from sterile anoxic stock solutions prepared under 80% N_2 and 20% CO_2 gas mixture. Adjust pH of complete medium to 6.9 - 7.0, if necessary.

Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
$MgSO_4$ x 7 H_2O	3.00	g
$MnSO_4$ x H_2O	0.50	g
NaCl	1.00	g
$FeSO_4$ x 7 H_2O	0.10	g
$CoSO_4$ x 7 H_2O	0.18	g
$CaCl_2$ x 2 H_2O	0.10	g
$ZnSO_4$ x 7 H_2O	0.18	g
$CuSO_4$ x 5 H_2O	0.01	g
$AlK(SO_4)_2$ x 12 H_2O	0.02	g
H_3BO_3	0.01	g
Na_2MoO_4 x 2 H_2O	0.01	g
$NiCl_2$ x 6 H_2O	0.03	g

© 2022 DSMZ - All rights reserved

Na_2SeO_3 x 5 H_2O	0.30	mg
Na_2WO_4 x 2 H_2O	0.40	mg
Distilled water	1000.00	ml

First dissolve nitrilotriacetic acid and adjust pH to 6.5 with KOH, then add minerals. Adjust final to pH 7.0 with KOH.

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

© 2022 DSMZ - All rights reserved