492: CLOSTRIDIUM METHYLPENTOSUM MEDIUM

Solution A	954.00	ml
Solution B	20.00	ml
Solution C	10.00	ml
Solution D	1.00	ml
Solution E	20.00	ml

Sparge solution A with 100% N_2 gas for at least 30 - 45 min to make it anoxic, then dispense medium under same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. Solution B is autoclaved under 80% N_2 and 20% CO_2 gas atmosphere. Solutions C and D are prepared under 100% N_2 gas atmosphere and sterilized by filtration. Solution E is autoclaved separately under 100% N_2 gas. Solutions B to E are added to the sterile solution A in the sequence as indicated. Final pH of the medium should be 6.8 - 7.0.

Solution A

NH_4Cl	0.90	g
KH_2PO_4	0.90	g
NaCl	0.90	g
$CaCl_2$ x 2 H_2O	20.00	mg
$MnCl_2$ x 4 H_2O	20.00	mg
$MgSO_4$ x 7 H_2O	20.00	mg
$CoCl_2$ x 6 H_2O (0.1% w/v)	5.00	ml
$FeSO_4$ x 7 H_2O (0.1% w/v in 0.1 N H_2SO_4)	5.00	ml
$ZnSO_4$ x 7 H_2O (0.1% w/v)	2.00	ml
$CuSO_4$ x H_2O (0.1% w/v)	2.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Distilled water	940.00	ml

Adjust pH to 6.5 with KOH.

Solution B

$NaHCO_3$	1.00	g
Distilled water	20.00	ml

Solution C

L-Rhamnose	2.00	g
Distilled water	10.00	ml

Solution D

Wolin's vitamin solution (10x)	1.00	ml

Solution E

L-Cysteine HCl x H_2O	1.00	g
Distilled water	20.00	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