492. CLOSTRIDIUM METHYLPENTOSUM MEDIUM

Solution A:

NH_4Cl	0.9	g
KH_2PO_4	0.9	g
NaCl	0.9	g
$CaCl_2$ x 2 H_2O	20.0	mg
$MnCl_2$ x 4 H_2O	20.0	mg
$MgSO_4$ x 7 H_2O	20.0	mg
$CoCl_2$ x 6 H_2O solution (0.1% w/v)	5.0	ml
$FeSO_4$ x 7 H_2O solution (0.1% w/v in 0.1 N H_2SO_4)	5.0	ml
$ZnSO_4$ x 7 H_2O solution (0.1% w/v)	2.0	ml
$CuSO_4$ x H_2O solution (0.1% w/v)	2.0	ml
Na-resazurin solution (0.1% w/v)	0.5	ml
Distilled water	940.0	ml

Adjust pH to 6.5 with KOH.

Solution B:

$NaHCO_3$	1.0	g
Distilled water	20.0	ml

Solution C:

L-Rhamnose	2.0	g
Distilled water	10.0	ml

Solution D:

Vitamin solution (see medium 141)	10.0	ml

Solution E:

L-Cysteine-HCl x H_2O	1.0	g
Distilled water	20.0	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.