1011c. DESULFOTHERMUS MJ MEDIUM (H2/CO2)

NaCl 30.00 g
K$_2$HPO$_4$ 0.14 g
CaCl$_2$ x 2 H$_2$O 0.14 g
MgSO$_4$ x 7 H$_2$O 3.40 g
MgCl$_2$ x 6 H$_2$O 4.18 g
KCl 0.33 g
NH$_4$Cl 0.25 g
Fe(NH$_4$)$_2$(SO$_4$)$_2$ x 6 H$_2$O 0.01 g
Trace element solution (see medium 141) 10.00 ml
Na$_2$CO$_3$ 1.50 g
Na-pyruvate 0.50 g
Na-lactate 0.50 g
Yeast extract 0.10 g
Na$_2$S$_2$O$_3$ x 5 H$_2$O 1.50 g
Vitamin solution (see medium 141) 10.00 ml
Distilled water 1000.00 ml

Dissolve ingredients (except carbonate, pyruvate, lactate, yeast extract, thiosulfate and vitamins), then sparge medium with 80% H$_2$ 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 up to a volume of 20% and autoclave. Add pyruvate, lactate, yeast extract, thiosulfate and vitamins to the autoclaved medium from sterile anoxic stock solutions prepared under 100% N$_2$ gas and carbonate from a sterile anoxic stock solution prepared under 80% N$_2$ and 20% CO$_2$ gas mixture. Solutions of vitamins and thiosulfate are sterilized by filtration. Adjust pH of the complete medium to 6.7. Prior to inoculation reduce medium with 10 - 20 mg/l sodium dithionite, added from a 5% (w/v) solution freshly prepared under N$_2$ and filter sterilized. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H$_2$ and 20% CO$_2$ gas mixture.

For DSM 22050 supplement medium with 1.00 g/l NaNO$_3$ and omit pyruvate, lactate and yeast extract. Upon autoclaving add 3.00 g/l sterile sulfur powder (sterilized by steaming for 3 hours on each of 3 successive days) and adjust pH to 6.0 – 6.5.

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For DSM 27205 supplement medium with 1.00 g/l NaNO₃ and omit pyruvate, lactate, yeast extract and dithionite. Upon autoclaving add 10.00 g/l sterile sulfur powder (sterilized by steaming for 3 hours on each of 3 successive days) and adjust pH to 6.0 – 6.5. After inoculation do not pressurize with 80% H₂ and 20% CO₂ gas mixture and add sterile air in an amount that is equivalent to a volume of 10% of the headspace.