

## 1011c. DESULFOTHERMUS MJ MEDIUM (H<sub>2</sub>/CO<sub>2</sub>)

NaCl	30.00	g
K <sub>2</sub> HPO <sub>4</sub>	0.14	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.14	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.40	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	4.18	g
KCl	0.33	g
NH <sub>4</sub> Cl	0.25	g
Fe(NH <sub>4</sub> ) <sub>2</sub> (SO <sub>4</sub> ) <sub>2</sub> x 6 H <sub>2</sub> O	0.01	g
Trace element solution (see medium 141)	10.00	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
Na-pyruvate	0.50	g
Na-lactate	0.50	g
Yeast extract	0.10	g
Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> x 5 H <sub>2</sub> 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<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub> gas and carbonate from a sterile anoxic stock solution prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> 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<sub>2</sub> and filter sterilized. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

For DSM 22050 supplement medium with 1.00 g/l NaNO<sub>3</sub> 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  $\text{NaNO}_3$  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%  $\text{H}_2$  and 20%  $\text{CO}_2$  gas mixture and add sterile air in an amount that is equivalent to a volume of 10% of the headspace.