

1011c: DESULFOTHERMUS MJ MEDIUM (H₂/CO₂)

This recipe contains strain-specific modifications for *Sulfurovum aggregans* DSM 27205 *

Final pH: * 6.0 - 6.5

Final volume: 1012 ml

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
Modified Wolin's mineral solution	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
Wolin's vitamin solution (10x)	1.00	ml
Na dithionite solution (5% w/v)	1.00	ml
Sulfur powder	10.00	g
NaNO ₃	1.00	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, pyruvate, lactate, yeast extract, thiosulfate, vitamins and dithionite), then sparge medium with 80% H₂ and 20% CO₂ 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% N₂ gas and carbonate and dithionite from sterile anoxic stock solutions prepared under 80% N₂ and 20% CO₂ gas mixture. Solutions of vitamins, thiosulfate and dithionite are sterilized by filtration. Adjust pH of the complete medium to 6.7.

2. After inoculation pressurize vessels to 2 bar overpressure with sterile 80% H₂ and 20% CO₂ gas mixture.

* 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.

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Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO ₄ x 7 H ₂ O	3.00	g
MnSO ₄ x H ₂ O	0.50	g
NaCl	1.00	g
FeSO ₄ x 7 H ₂ O	0.10	g
CoSO ₄ x 7 H ₂ O	0.18	g
CaCl ₂ x 2 H ₂ O	0.10	g
ZnSO ₄ x 7 H ₂ O	0.18	g
CuSO ₄ x 5 H ₂ O	0.01	g
AlK(SO ₄) ₂ x 12 H ₂ O	0.02	g
H ₃ BO ₃	0.01	g
Na ₂ MoO ₄ x 2 H ₂ O	0.01	g
NiCl ₂ x 6 H ₂ O	0.03	g
Na ₂ SeO ₃ x 5 H ₂ O	0.30	mg
Na ₂ WO ₄ x 2 H ₂ O	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

Na-dithionite solution (5% w/v) (from medium 829)

NaHCO ₃	50.00	g
Na ₂ S ₂ O ₄	50.00	g
Distilled water	1000.00	ml

Dissolve NaHCO₃ in water and make the solution anoxic by sparging with 80% N₂ and 20% CO₂ gas mixture. Then dissolve the Na-dithionite and filter sterilize the solution into anoxic Hungate tubes. Store the prepared solution in the dark and refrigerated. Prepare only small amounts of stock solution, as Na-dithionite decomposes rapidly.