

165: ACETIVIBRIO CELLULOLYTICUS MEDIUM

Mineral solution 1	75.00	ml
Mineral solution 2	75.00	ml
Modified Wolin's mineral solution	10.00	ml
FeSO₄ x 7 H₂O solution (0.1% w/v)	20.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Cellobiose	3.00	g
Cellulose (MN300) (optional)	3.00	g
Wolin's vitamin solution (10x)	1.00	ml
L-Cysteine HCl x H ₂ O	0.25	g
Na ₂ S x 9 H ₂ O	0.25	g
Distilled water	820.00	ml

Dissolve ingredients except carbonate, cellobiose, vitamins, cysteine and sulfide, then sparge medium with 80% N₂ and 20% CO₂ gas mixture for 30 - 45 min to make it anoxic. Dispense medium under the same gas atmosphere into anoxic Hungate-type tubes and autoclave. Add cellobiose, vitamins, cysteine and sulfide from sterile anoxic stock solutions prepared under 100% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ gas mixture. Cellobiose and vitamins have to be sterilized by filtration. Prior to use adjust pH of complete medium to 7.2 - 7.4. Cellulose (MN 300, Whatman CF II, Kleenex tissue paper, or HCl treated cotton) can be used as an alternative to cellobiose.

Mineral solution 1

K ₂ HPO ₄	3.90	g
Distilled water	1000.00	ml

Mineral solution 2

KH ₂ PO ₄	2.40	g
(NH ₄) ₂ SO ₄	6.00	g
NaCl	0.59	g
MgSO ₄ x 7 H ₂ O	1.20	g
CaCl ₂ x 2 H ₂ O	0.72	g
Distilled water	1000.00	ml

Modified Wolin's mineral solution (from medium 141)

Nitrolotriacetic 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.

FeSO₄ x 7 H₂O solution (0.1% w/v) (from medium 119)

FeSO ₄ x 7 H ₂ O	1.00	g
H ₂ SO ₄ (0.1 N)	1000.00	ml

The ferrous sulfate solution is not stable and should be freshly prepared.

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