

## 135: ACETOBACTERIUM MEDIUM

This recipe contains strain-specific modifications for *Acetobacterium malicum* DSM 4132 \*

NH <sub>4</sub> Cl	1.00	g
KH <sub>2</sub> PO <sub>4</sub>	0.33	g
K <sub>2</sub> HPO <sub>4</sub>	0.45	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
<b>Modified Wolin's mineral solution</b>	20.00	ml
Yeast extract	2.00	g
Sodium resazurin (0.1% w/v)	0.50	ml
NaHCO <sub>3</sub>	10.00	g
<b>D-Fructose</b>	<b>1.00</b>	<b>g</b>
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
L-Cysteine HCl x H <sub>2</sub> O	0.50	g
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
Distilled water	1000.00	ml

1. Dissolve ingredients except bicarbonate, fructose, vitamins, cysteine and sulfide, bring to the boil and cool to room temperature under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture. Add bicarbonate (solid) and equilibrate the medium with the gas until a pH of around 7.4 is reached. Then distribute under the same gas atmosphere in anoxic Hungate-type tubes or serum vials and autoclave. Before use adjust the pH to 8.0 - 8.2 by adding a sterile anoxic stock solution of sodium carbonate (5% w/v) prepared under 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture (c. 0.25 ml per 10 ml medium) and add fructose, vitamins (sterilized by filtration), cysteine and sulfide from anoxic sterile stock solutions prepared under 100% N<sub>2</sub>.

2. Note: For autotrophic growth D-fructose is omitted and a gas atmosphere of 80% H<sub>2</sub> and 20% CO<sub>2</sub> is used.

\* Use D-fructose as substrate at a final concentration of 1.00 g/l.

### Modified Wolin's mineral solution (from medium 141)

Nitrilotriacetic acid	1.50	g
MgSO <sub>4</sub> x 7 H <sub>2</sub> O	3.00	g
MnSO <sub>4</sub> x H <sub>2</sub> O	0.50	g
NaCl	1.00	g
FeSO <sub>4</sub> x 7 H <sub>2</sub> O	0.10	g
CoSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.10	g
ZnSO <sub>4</sub> x 7 H <sub>2</sub> O	0.18	g
CuSO <sub>4</sub> x 5 H <sub>2</sub> O	0.01	g
AlK(SO <sub>4</sub> ) <sub>2</sub> x 12 H <sub>2</sub> O	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g

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Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	0.01	g
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	0.03	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	0.30	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> 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 <sub>12</sub>	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml