

## 870: SYNTROPHOTHERMUS MEDIUM

This recipe contains strain-specific modifications for *Syntrophothermus lipocalidus* DSM 12681

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KH <sub>2</sub> PO <sub>4</sub>	0.14	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.20	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.15	g
NH <sub>4</sub> Cl	0.54	g
<b>Modified Wolin's mineral solution</b>	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
<del>Na-crotonate solution (1 M)</del>	<del>10.00</del>	<del>ml</del>
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.30	g
L-Cysteine HCl x H <sub>2</sub> O	0.30	g
Na-butyrate	2.20	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, crotonate, vitamins, sulfide and cysteine, then sparge medium with 80% N<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 and autoclave. Add crotonate, vitamins, sulfide and cysteine 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 atmosphere. Sterilize vitamins by filtration. Adjust pH of complete medium to 7.0, if necessary.

\* Replace Na-crotonate with 2.20 g/l Na-butyrate.

### 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
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

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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.

### **Na-crotonate solution (1 M)** (from medium 870)

Crotonic acid (ALDRICH 113018)	86.00	g
NaOH (10 N)	100.00	ml
Distilled water	900.00	ml

Dissolve crotonic acid in 800 ml distilled water, add around 100 ml of 10 N NaOH and adjust pH to around 7. Then add water to reach a volume of 1000 ml. Sterilize by filtration under 100% N<sub>2</sub> gas atmosphere.

### **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