

## **870: SYNTROPHOTHERMUS MEDIUM**

KH <sub>2</sub> PO <sub>4</sub>	0.14	g
$MgCl_2 \ge 6 H_2O$	0.20	g
$CaCl_2 \times 2 H_2O$	0.15	g
NH <sub>4</sub> Cl	0.54	g
Modified Wolin's mineral solution	1.00	ml
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.00	g
Na-crotonate solution (1 M)	10.00	ml
Wolin's vitamin solution (10x)	1.00	ml
$Na_2S \times 9 H_2O$	0.30	g
L-Cysteine HCl x $H_2O$	0.30	g
Distilled water	1000.00	ml

Dissolve all ingredients except carbonate, crotonate, vitamins, sulfide and cysteine, then sparge medium with 80%  $N_2$  and 20%  $CO_2$  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%  $N_2$  gas and carbonate from a sterile anoxic stock solution prepared under 80%  $N_2$  and 20%  $CO_2$  gas atmosphere. Sterilize vitamins by filtration. Adjust pH of complete medium to 7.0, if necessary.

For <u>DSM 12681</u>: Replace Na-crotonate with 2.20 g/l Na-butyrate.

Nitrilotriacetic acid	1.50	g
$MgSO_4 \times 7 H_2O$	3.00	g
$MnSO_4 \times H_2O$	0.50	g
NaCl	1.00	g
$FeSO_4 \times 7 H_2O$	0.10	g
$CoSO_4 \times 7 H_2O$	0.18	g
$CaCl_2 \times 2 H_2O$	0.10	g
$ZnSO_4 \times 7 H_2O$	0.18	g
$CuSO_4 \times 5 H_2O$	0.01	g
$AIK(SO_4)_2 \times 12 H_2O$	0.02	g
H <sub>3</sub> BO <sub>3</sub>	0.01	g
$Na_2MoO_4 \ge H_2O$	0.01	g
$NiCl_2 \times 6 H_2O$	0.03	g
$Na_2SeO_3 \times 5 H_2O$	0.30	mg
$Na_2WO_4 \ge H_2O$	0.40	mg
Distilled water	1000.00	ml

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



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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%  $N_2$  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 HCI	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