

## 684: SYNTROPHOBACTER (MPOB) MEDIUM

This recipe contains strain-specific modifications for *Methanomethylovorans thermophila* DSM 17232 \*

Final pH: 7.0 - 7.2

Final volume: 1003 ml

Na <sub>2</sub> HPO <sub>4</sub> x 2 H <sub>2</sub> O	0.53	g
KH <sub>2</sub> PO <sub>4</sub>	0.41	g
NH <sub>4</sub> Cl	0.30	g
CaCl <sub>2</sub> x 2 H <sub>2</sub> O	0.11	g
MgCl <sub>2</sub> x 6 H <sub>2</sub> O	0.10	g
NaCl	0.30	g
<b>Trace element solution SL-10</b>	1.00	ml
<b>Selenite-tungstate solution</b>	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na <sub>2</sub> CO <sub>3</sub>	1.50	g
<del>Na<sub>2</sub>-fumarate</del>	<del>3.20</del>	<del>g</del>
<b>Wolin's vitamin solution (10x)</b>	1.00	ml
Na <sub>2</sub> S x 9 H <sub>2</sub> O	0.50	g
<b>Methanol</b>	<b>2.00</b>	<b>ml</b>
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, fumarate and sulfide) and 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. After autoclaving complete the medium by adding vitamins, fumarate and sulfide 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. Stock solutions of vitamins and fumarate are sterilized by filtration. Adjust pH of the complete medium to 7.0 - 7.2. After inoculation pressurize culture vials to 0.7 bar overpressure with sterile 80% N<sub>2</sub> and 20% CO<sub>2</sub> gas mixture.

2. Note: A white precipitate forms in this medium after autoclaving, which has however no negative effect on growth.

\* Replace Na<sub>2</sub>-fumarate with 2.00 ml/l methanol added after autoclaving from a sterile anoxic stock solution prepared under 100% N<sub>2</sub> gas atmosphere.

### Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl <sub>2</sub> x 4 H <sub>2</sub> O	1.50	g
ZnCl <sub>2</sub>	70.00	mg

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MnCl <sub>2</sub> x 4 H <sub>2</sub> O	100.00	mg
H <sub>3</sub> BO <sub>3</sub>	6.00	mg
CoCl <sub>2</sub> x 6 H <sub>2</sub> O	190.00	mg
CuCl <sub>2</sub> x 2 H <sub>2</sub> O	2.00	mg
NiCl <sub>2</sub> x 6 H <sub>2</sub> O	24.00	mg
Na <sub>2</sub> MoO <sub>4</sub> x 2 H <sub>2</sub> O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl<sub>2</sub> in the HCl, then dilute in water, add and dissolve the other salts. Finally make up to 1000.00 ml.

### Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na <sub>2</sub> SeO <sub>3</sub> x 5 H <sub>2</sub> O	3.00	mg
Na <sub>2</sub> WO <sub>4</sub> x 2 H <sub>2</sub> O	4.00	mg
Distilled water	1000.00	ml

### 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