

684: SYNTROPHOBACTER (MPOB) MEDIUM

This recipe contains strain-specific modifications for *Ercella succinigenes* DSM 27333 *

Final pH: * 7.5

Final volume: 1003 ml

Na ₂ HPO ₄ x 2 H ₂ O	0.53	g
KH ₂ PO ₄	0.41	g
NH ₄ Cl	0.30	g
CaCl ₂ x 2 H ₂ O	0.11	g
MgCl ₂ x 6 H ₂ O	0.10	g
NaCl	0.30	g
Trace element solution SL-10	1.00	ml
Selenite-tungstate solution	1.00	ml
Yeast extract	0.20	g
Sodium resazurin (0.1% w/v)	0.50	ml
Na ₂ CO ₃	1.50	g
Na₂ fumarate	3.20	g
Wolin's vitamin solution (10x)	1.00	ml
Na ₂ S x 9 H ₂ O	0.50	g
Glycerol	2.30	g
Distilled water	1000.00	ml

1. Dissolve ingredients (except carbonate, vitamins, fumarate and sulfide) and sparge medium with 80% N₂ and 20% CO₂ 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% N₂ gas and carbonate from a sterile anoxic stock solution prepared under 80% N₂ and 20% CO₂ 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% N₂ and 20% CO₂ gas mixture.

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

* Replace Na₂-fumarate with 2.30 g/l glycerol added after autoclaving from a sterile anoxic solution prepared under 100% N₂ gas atmosphere. Adjust pH of complete medium to 7.5.

Trace element solution SL-10 (from medium 320)

HCl (25%)	10.00	ml
FeCl ₂ x 4 H ₂ O	1.50	g
ZnCl ₂	70.00	mg
MnCl ₂ x 4 H ₂ O	100.00	mg

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H ₃ BO ₃	6.00	mg
CoCl ₂ x 6 H ₂ O	190.00	mg
CuCl ₂ x 2 H ₂ O	2.00	mg
NiCl ₂ x 6 H ₂ O	24.00	mg
Na ₂ MoO ₄ x 2 H ₂ O	36.00	mg
Distilled water	990.00	ml

First dissolve FeCl₂ 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 ₂ SeO ₃ x 5 H ₂ O	3.00	mg
Na ₂ WO ₄ x 2 H ₂ 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 ₁₂	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml