

AMB-1 Growth Guidelines

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Modified By Penn iGEM 2014

Goal: to grow AMB-1 in liquid culture and solid culture, to make growth media

Protocol:

As a general guide to how this tube would be propagated for a short-term experiment we would suggest:

Inoculate cells from 1.7 ul tube into a 10 ml culture (1:100 dilution) with MG liquid media (see recipe below)

Grow culture at 4% O₂, 30°C overnight

As a general guide to how this tube would be propagated for longer-term experimentation we would suggest:

Inoculate cells from 1.7 ul tube into a 50 ml sterile falcon tube (1:100 dilution) with MG liquid media + Vitamins and Iron (see recipe below)

Grow 50 ml culture for 2 days at 30°C (no need for 4% O₂ as depletion in full falcon tube is sufficient)

Spin down cells (8000 X g at room temperature for 10 minutes)

Create glycerol stock (30% glycerol final)

Then, one would streak out cells onto MG Agar plates

Plates require 4% O₂, 30°C for 5-7 days to form single colonies

Pick colonies into 1.7 ml tubes with MG media

Materials:

AMB-1 Growth Media

- Fill a beaker with MQ water to 90% of final volume
- Add the chemicals in the specified order.
- Do not mix all chemicals in powder form to then add the water. This seems to cause a problem for cell growth which we haven't figured out yet.

Chemical	per 1L (g)	per 4L (g)	Supplier
L-Ascorbic acid	0.035	0.14	Sigma, Cat.# A92902
Sodium acetate	0.07	0.28	Sigma, Cat.# S7545
Sodium thiosulfate	0.1	0.4	Sigma, Cat.# S7026
Sodium nitrate	0.12	0.48	Sigma, Cat.# 221341
Succinic acid	0.37	1.48	Sigma, Cat.# 398055
L-Tartaric acid	0.37	1.48	Sigma, Cat.# T109
Potassium phosphate monobasic	0.68	2.72	Sigma, Cat.# P9791
Wolfe's Mineral solution (-Fe)	5 ml	20 ml	"Home made"

- **pH solution to 6.9 with 10M NaOH**
- **For agar plates (0.7%) add:**
2.8g of Agar per liter (Sigma, Cat. # A1296)
- **Autoclave for 25 min.**

NOTES:

The recipe above is to prepare the basic solution of the MG medium. Vitamins and an iron source are missing from this and are added after autoclaving and just before starting cultures. For agar plates: let the medium cool down to ~45°C (hand warm) before adding additional components.

The following components are at a 100X concentration:

- 1) Wolfe's vitamins solution
- 2) Ferric malate

Wolfe's mineral solution:

Add nitrilotriacetic acid to approximately 500 mL of water and adjust pH to 6.5 with KOH to dissolve. Bring volume to 1L with remaining MQ water and add the following compounds one at a time (all reagents are from Sigma-Aldrich).

NOTE: You won't be able to accurately weigh out most of the chemicals. To avoid errors make 4 L instead of 1 L.

No need to sterilize. Wrap bottle with aluminium foil to protect from light exposure and store at room temperature. Good for a long time

Chemical	Cat. #	Amount per 1L
Nitrilotriacetic acid	N0128	1.5 g
MgSO ₄	M7506	1.465 g
MnSO ₄ · H ₂ O	M7634	0.5 g
NaCl	S6191	1.0 g
CoCl ₂ · 6 H ₂ O	255599	0.1 g
CaCl ₂ · 2 H ₂ O	C5080	0.1 g
ZnSO ₄ · 7 H ₂ O	Z4750	0.1 g
CuSO ₄ · 5 H ₂ O	C7631	0.01 g

$\text{AlK}(\text{SO}_4)_2 \cdot 12 \text{H}_2\text{O}$	A7210	0.01 g
H_3BO_3	B0394	0.01 g
$\text{Na}_2\text{MoO}_4 \cdot 2 \text{H}_2\text{O}$	M1003	0.01 g

Solution also available from ATCC (Cat. # MD-TMS; ~\$65 for 50 ml)

Ferric malate 100X stock solution

3 mM Fe
9 mM Malate

Chemical	per 1L (g)	Supplier
Iron(III)chloride	0.486	Sigma Cat # F7134
DL-Malic acid	1.207	Sigma Cat # M0875

Note: Make with MQ water. Filter sterilize solution by filtration through a Nalgene filter with 0.22 um pore size. Wrap the container in which the solution is kept with aluminium foil to prevent light exposure and store at RT. Solution should be stable for long time.

Wolfe's vitamin solution:

NOTE: you won't be able to accurately weigh out most of the chemicals. To avoid errors make up 4 L instead of 1 L.

Chemical	Supplier / Cat. #	Amount per 1L
Biotin	Sigma, B4501	2 mg
Folic acid	Sigma, 7876	2 mg
Pyridoxine hydrochloride	Fluka, 95180	10 mg
Vitamin B1 HCl	Fluka, 95160	5 mg
(-)-Riboflavin	Sigma, R9504	5 mg
Nicotinic acid	Fluka, 72309	5 mg
Calcium pantothenate	Sigma, C8731	5 mg
Vitamin B12	Sigma, V2876	0.1 mg
4-Aminobenzoic acid	Sigma, A9878	5 mg

(±)- α -Lipoic acid	Sigma, T5625	5 mg
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Solution is also available through ATCC (Cat. # MD-VS; ~\$65 for 50 ml).