



## Continuous culture of *Lemna minor*

### Principle

This protocol describes the timing and proceedings to maintain a healthy stock of *Lemna minor*.

### Equipment and Reagents

Machine/Product	Reference (Company, Type, ...)
Autoclave	
Sterile Erlenmeyer of 250mL	
Sterile 100 ml Graduated Cylinder	
hydrophobic cotton	
aluminium foil	
Fresh stock culture	
Laminar Flow Hood	
Flammable or sterile disposable inoculating loops	
Ummonium 38	 LAB. HUCKERT'S INTERNATIONAL
Ethanol 99% denaturated with propanol	 VWR International #87137

### Protocol

To think about:

- Sterilize enough Erlenmeyer flasks of 250mL closed with hydrophobic cotton and aluminium foil
- Switch on the laminar flow about 30 min before you start on full power
- clean out flow with umonium
- Wear gloves and disinfect your hands with ethanol every time you go in or out of the flow
- Spray the bottles always with ethanol when you put them in the flow
- Flame your bottles before and after you used them
- Take care you don't disrupt the flow of sterile air, always keep your bottles directed towards the air flow and avoid going with your hands over the bottles
- Avoid contact of the cotton with the laminar flow work surface

### Growth Conditions and timing

Stock culture grown at 21°C with continuous light ( $\pm 50\mu\text{mol/s.m}^2$ ) are being transferred every 10 to 12 days

3 *Lemna* plants of 3-4 fronts are transferred into 100mL of medium

Take care: only use healthy stock cultures (plants are all consisting of 3-4 or more fronds, older fronds are dark green whereas young fronds are light green, medium is clear, roots are long)

## Growth Medium for Lemna minor

### Stock solution

		Stock solution g/L	mL for 1 Liter stock
I	KNO <sub>3</sub>	10 g/ <b>1 L</b>	10 <b>30</b>
	Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	24 g / <b>1 L</b>	24
II	MgSO <sub>4</sub> ·7H <sub>2</sub> O	24,6 g/100mL	246 3
III	KH <sub>2</sub> PO <sub>4</sub>	13,8g/100mL	138 3
IV	ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1 g/L	1 1
	MnSO <sub>4</sub> ·H <sub>2</sub> O	100 mg/L	0,1
	CuSO <sub>4</sub> ·5H <sub>2</sub> O	30 mg/L	0,03
	Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	100 mg/L	0,1
	Ferric citrate	1 g/L	1
	Na-EDTA	2,9 g/L	2,9
	H <sub>3</sub> BO <sub>3</sub>	1g/L	1

Composition of Hutner's medium			
	concentration mg/L	Molar mass g/mol	concentration μM
KNO <sub>3</sub>	300	101,1	2967,16
Ca(NO <sub>3</sub> ) <sub>2</sub> ·4H <sub>2</sub> O	720	236,2	3048,90
MgSO <sub>4</sub> ·7H <sub>2</sub> O	738	246,5	2994,29
KH <sub>2</sub> PO <sub>4</sub>	414	136,1	3042,12
ZnSO <sub>4</sub> ·7H <sub>2</sub> O	1	287,5	3,48
MnSO <sub>4</sub> ·H <sub>2</sub> O	0,1	169,0	0,59
CuSO <sub>4</sub> ·5H <sub>2</sub> O	0,03	159,6	0,19
Na <sub>2</sub> MoO <sub>4</sub> ·2H <sub>2</sub> O	0,1	242,0	0,41
Ferric citrate	1	244,9	4,08
Na-EDTA	2,9	372,2	7,79
H <sub>3</sub> BO <sub>3</sub>	1	61,8	16,17