

Supplementary information

Do-it-yourself, stand-alone, open-source Nebulizer for derivatizations and bioassays in planar chromatography

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Table S1: Bill of materials

Parts	Amount	Exemplary supplier	Euro (+VAT)
Nebulizer casing			
Glass plate, 200 mm x 200 mm x 1 mm (or cost-free: a recycled used glass plate with layer washed-off)	1	ebay.de (Glasludwig)	4.17
Ultrasonic Piezo Atomizer Disc, Ø 16 mm, 108 kHz, 5 µm pore size, 30 cm cable JST PH 2.0 (model PZT-16-108K)	50	piezoelements.com	100.00 ¹
Ultrasonic Piezo Atomizer Disc, Ø 16 mm, 108 kHz, 11 µm pore size, 30 cm cable JST PH 2.0 (model PZT-16-108K)	50	piezoelements.com	100.00 ¹
3D Filament, PLA, 580 g needed	1	filamentworld.de	12.13 ²
3D Filament, PA12, Handtmann-Lauramid, 50 g sample (31 g needed for 2 atomizer holders)	1	filamentworld.de	9.16
		Sum	225.46
Nebulizer control unit			
Control board, electronic parts (see KiCad files and Mouser-basket)	1	Mouser	45.31
Control board, printed circuit board (PCB), upload 'gerber.zip'	5	https://jlcpcb.com	1.92 ³
Power supply, 12 V, 1.5 A, plug 5.5/2.5 mm (Amazon No. B0BRQHJ3Z7)	1	Amazon	5.87
USB 2.0 A-Male to Mini-B Cable (Amazon No. B004RPK91E)	1	Amazon	1.23
Screw M3*10	3	Local workshop	0.30
Self-Adhesive Elastic Buffer Square 12.5 x 12.5 mm, Height: 5.8 mm, Black (Pack of 40) (Amazon no. B06X3T5Z3D)	1	Amazon, Local workshop	7.13
3D-Filament, PLA, 85 g needed	1	filamentworld.de	1.78 ²
		Sum	63.54
Exhaust unit			
Hose coupling, 4-5 mm tube (Amazon No. B08PQ3W5KV)	1	Amazon	2.68
Silicone tube, 4 mm x 6 mm, 1 m (Amazon No. B08CQGB9JX)	1	Amazon	3.53
DEWIN Micro Vacuum Pump, 12 V (Amazon No. B07DVBQJCR)	1	Amazon	5.20
DC Chassis Mount Socket, 5.5/2.5 mm (Digikey No. CP-6-ND)	1	Digikey	1.67
Cable Assembly, 2.5mm ID, 5.5mm OD, Plug to Wire Leads (1.83m) (Digikey No. 839-1165-ND)	1	Digikey	4.61
Extension spring, 27.7 mm => 80 mm (Sodemann No. 30910)	1	sodemann-federn.de	4.60
Screw top bottle, 250 ml, GL 45 (Amazon No. B0C1BC3HTG)	1	Amazon	9.23
3D Filament, PLA, 155 g needed	1	filamentworld.de	3.24 ²
		Sum	34.76
		Sum in total	323.76
¹ Minimum order quantity 50 (custom made products, 20 cm cables), ask for atomizers in stock (minimum order quantity 10).			
The 11 µm pore size atomizers are needed to nebulize suspensions of larger yeast cells.			
Ultrasonic Piezo Atomizer 16 mm 108 kHz 5 µm pore size also are available at Amazon (amazon.de, product ASIN B0DFGH691W),			
one pack of 10 costs 10.08 €, but need a cable extension of about 15 cm			
² Calculated on 20.92 €/kg (filamentworld.de)			
³ At jlcpcb.com, upload 'gerber.zip' (board layout) and select 'Global Standard Direct Line' for shipping: 5.84 €; minimum order quantity 5			

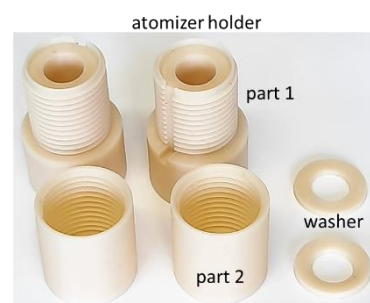
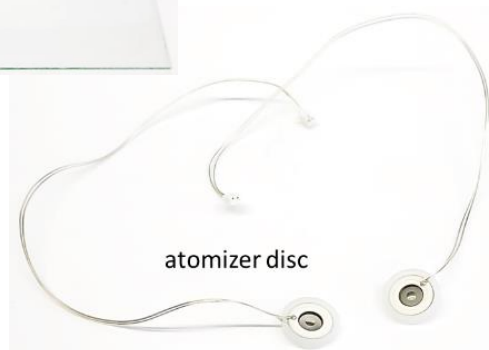
Instruction S1 Self-assembly of the Nebulizer 20 cm x 10 cm

The Nebulizer 20 cm x 10 cm consists of three parts:

- I. 3D-printed casing
- II. Exhaust unit
- III. Control unit for stand-alone use

I. 3D-printed casing

Materials	Qty
Glass plate 200 mm x 200 mm x 1 mm	1
Ultrasonic Piezo Atomizer Disc, Ø 16 mm, 108 kHz, 5 µm pore size	2
Ultrasonic Piezo Atomizer Disc, Ø 16 mm, 108 kHz, 11 µm pore size	2
JST PH2 Pin cable male header 2 pins, 20 cm	2
3D-printed nebulizer_base (PLA)	1
3D-printed nebulizer_extension (PLA)	1
3D-printed nebulizer_lid (PLA)	1
3D-printed nebulizer_atomizer_holder_part-1 (PA12)	2
3D-printed nebulizer_atomizer_holder_part-2 (PA12)	2
3D-printed nebulizer_atomizer_holder_washer (PA12)	2



3D-print instructions

The PLA parts were printed on a Smooth PEI Steel Sheet with standard slicer settings; supports on build plate only for the extension.

The PA12 parts were printed on a PA Nylon Powder-coated Steel Sheet. A Textured Powder-coated Steel Sheet with glue stick can also be used. Slicer settings: 0.1 mm layer height, infill 15 % (gyroid), combine infill every 2 layers, top and bottom infill pattern concentric, no supports.

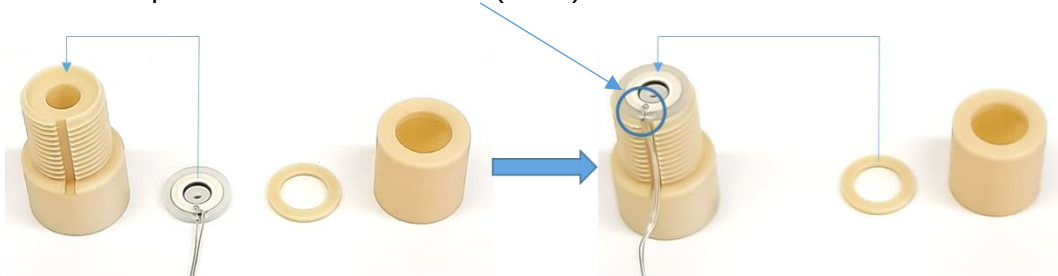
Assembly instruction

- Insert the glass plate into the slit of the nebulizer_base; clean the slit before from printing residues.

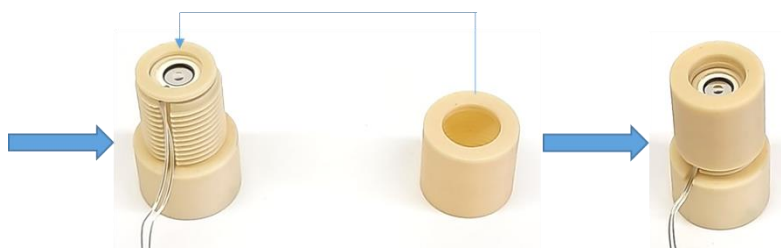


- Place the nebulizer_extension on top of the nebulizer_base, and the nebulizer_lid on top of the extension.

- Insert the atomizer disc into the atomizer_holder_part-1, so that the solder pad and the wires point to the cable channel (circle).



- Place the washer onto the atomizer disc and tightly screw the atomizer_holder_part-2 onto the part-1. Be careful guiding the wires and do not pull on the wires!



- Insert the mounted atomizer holders into the round cutouts of the nebulizer_lid.

The atomizers can simply be connected to the 2LabsToGo-Eco mainboard.

- Connect two JST PH2 Pin cables to the respective plug to be inserted into the mainboard.
- Connect the atomizer discs to the JST headers.

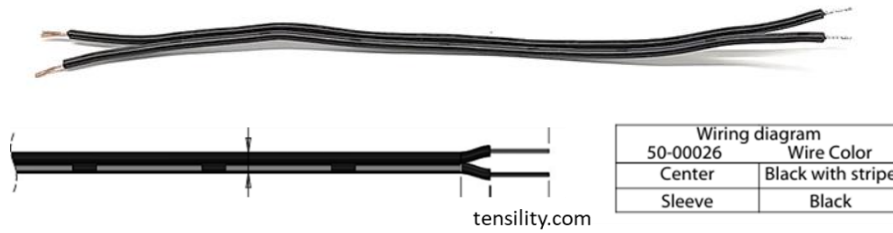


II. Exhaust unit

Materials	Qty
Glass bottle, 250 ml, GL 45 screw cap	1
Micro Vacuum Pump, 12 V	1
Silicone tube, 4 mm x 6 mm, 1 m	1
Hose coupling, 4-5 mm tube	1
DC Chassis Mount Socket, 5.5/2.5 mm	1
Cable Assembly, 2.5mm ID, 5.5mm OD, Plug to Wire Leads (1.83m)	1
Extension spring, 30 mm => 80 mm	1
3D-printed nebulizer_exhaust-unit_base	1
3D-printed nebulizer_exhaust-unit_pump-holder	1
3D-printed nebulizer_exhaust-unit_lid	1
3D-printed nebulizer_hose_mount	1

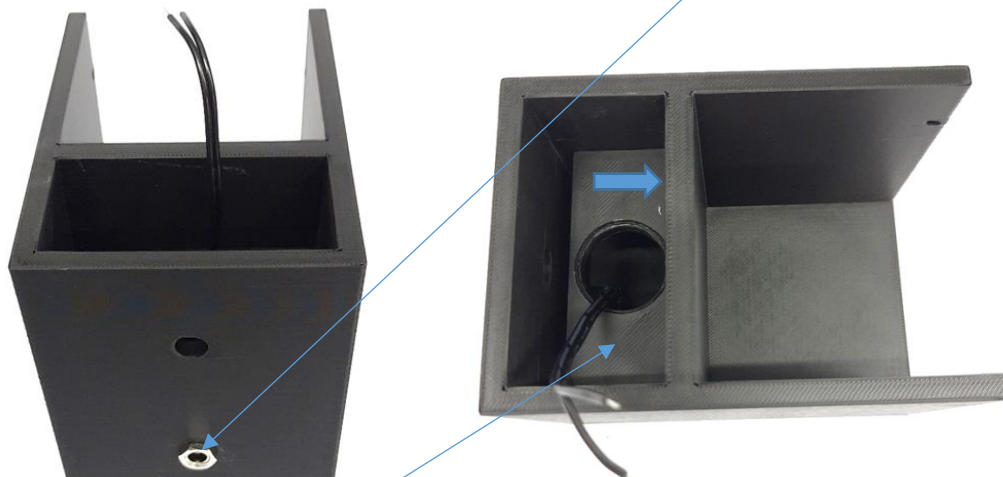


- From the end of the cable assembly, cut 15 cm and strip 5 mm from both wires. The black wire with the stripes is (+).



[The rest of the cable assembly is used for the control unit.]

- Solder the black wire with the stripes (+) to the center pin of the DC Chassis Mount Socket and the black wire to the sleeve (-).
- Insert the socket from inside the exhaust-unit_base into the round cutout near the bottom and fix it with the screw.

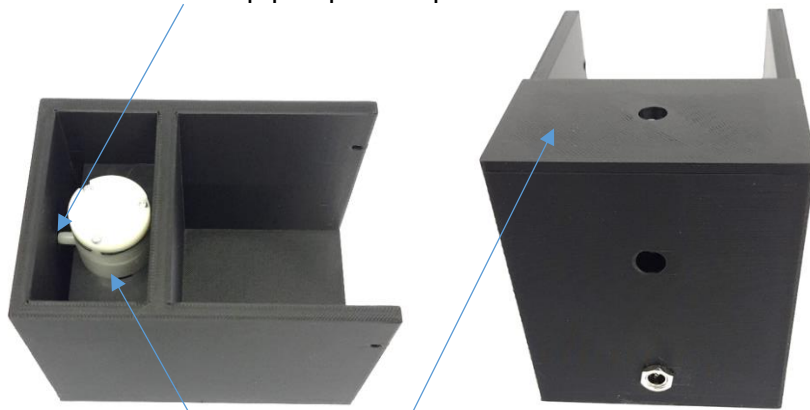


- Insert the pump-holder into the exhaust-unit_base and push it down and towards the middle wall while guiding the wires through the hole upwards.



- Turn and place the vacuum pump top-down into the exhaust-unit_base and solder the two wires to the solder tags. There is a red mark on the pump (circle) marking + (wire with the stripes).

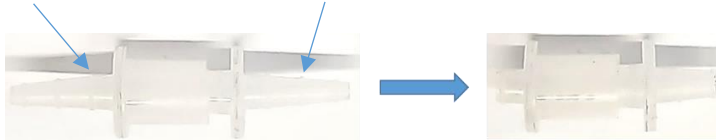
- Insert the vacuum pump into the pump-holder while guiding the wires under the pump-holder. The vacuum pump outlet points to the round cutout on the left side.



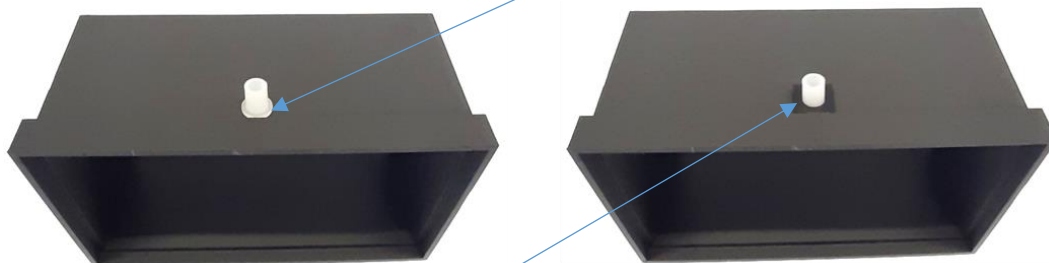
- Fix the pump with two drops of glue on both sides.
- Press the lid onto the exhaust_base.
- Drill two holes of 6 mm into the screw cap of the 250-mL bottle, and screw the cap onto the bottle.
- From the silicone tube, cut 30 cm; press one end onto the out-port of the vacuum pump and guide the other end through the cap to the bottom of the bottle. For the use, fill 100 mL of water into the bottle.
- Connect the rest of the silicone tube to the in-port of the vacuum pump.
- Mount the extension spring in the two holes on the open side to fix the bottle.



- Cut the hose coupling after the first ring and after the second ring of the olives.



- Disconnect the hose coupling and press the short olive into the round cutout on the back of the extension.

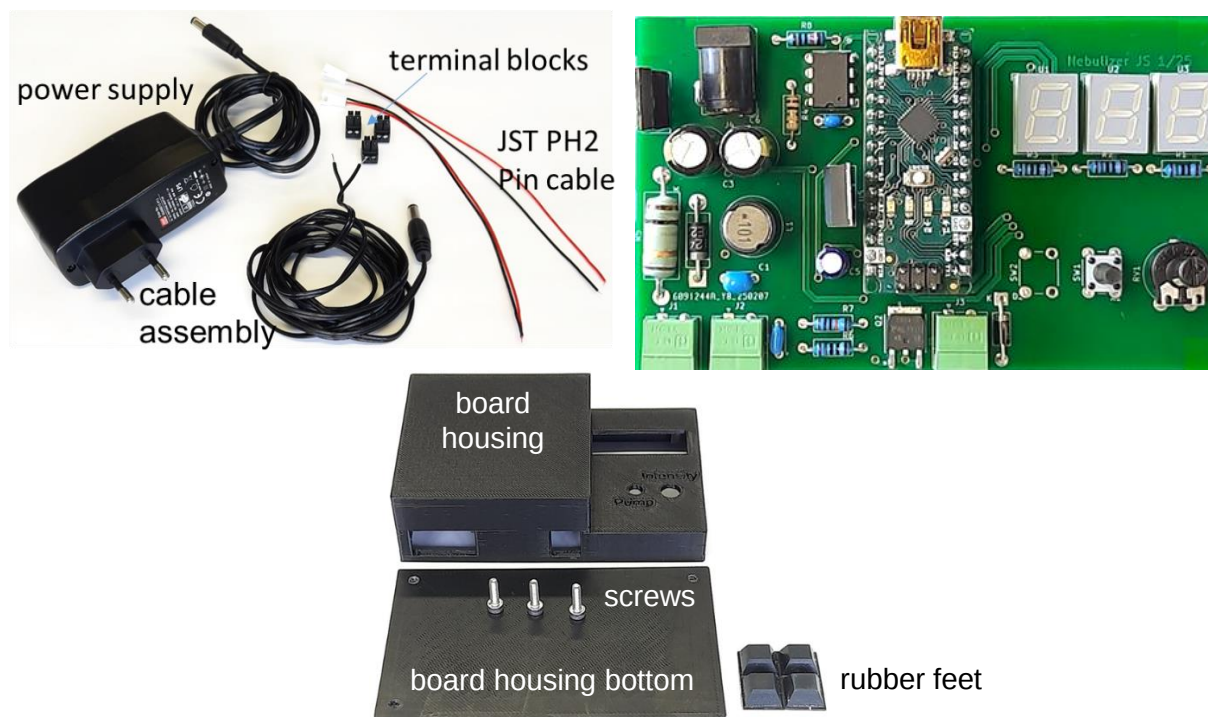


- Glue the 3D-printed hose_mount onto the extension to fix the hose coupling [polypropylen cannot be glued directly].
- Connect the long silicone tubing to the other part of the hose coupling. This way the exhaust unit can be connected/disconnected to/from the Nebulizer 20 cm x 10 cm.

The exhaust unit (vacuum pump) can separately be powered by a 12 V (1 A) power supply or by using the Control unit described next.

III. Control unit for stand-alone use

Materials	Qty
Electronic board	1
M3x10 screws, DIN 912	3
Self-adhesive rubber feet	4
2-Position terminal block	3
Power supply 12 V, 1.5 A, plug 2.5 mm x 5.5 mm	1
Cable Assembly, 2.5mm ID, 5.5mm OD, Plug to Wire Leads (1.83m)	1
3D-printed nebulizer_board_housing	1
3D-printed nebulizer_board_housing_bottom	1
JST PH2 Pin cable male header 2 pins, 20 cm → already listed in I. 3D-printed casing	2



3-D Printing instruction

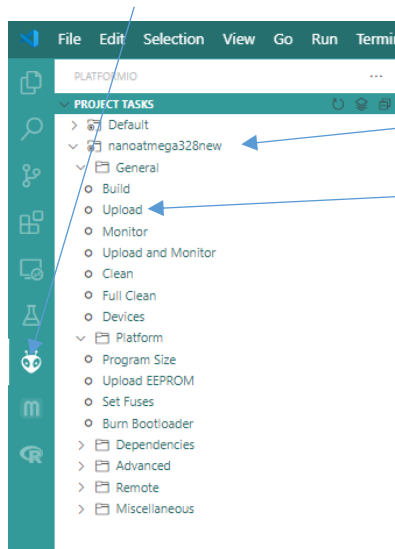
The 3D-parts were printed with PLA and standard printer settings. The housing needs support material everywhere, but support should be blocked in the three screw holes of the nebulizer_board_housing.

Uploading the firmware

- Download the GitHub repository <https://github.com/OfficeChromatography/Nebulizer20x10> and unzip the downloaded file.

The easiest way to upload the firmware onto the Arduino nano is to use Visual Studio Code, freely available at <https://code.visualstudio.com/download>.

- Connect the board by an “USB 2.0 A-Male to Mini-B cable” to a PC and wait to be recognized.
- Start Visual Studio Code and, in the File menu, open the unzipped folder “Firmware”.
- Wait until the PlatformIO processes are finished.
- Click the PIO button.



The board is displayed here.

Click upload to upload the firmware.

Success is reported in the terminal like this:

```

Reading | ##### | 100% 1.03s

avrdude: verifying ...
avrdude: 3068 bytes of flash verified

avrdude: safemode: Fuses OK (E:00, H:00, L:00)

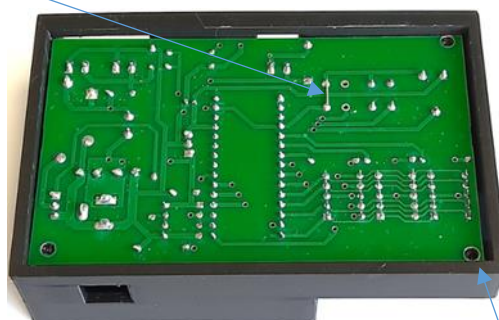
avrdude done. Thank you.

```

- Disconnect the board.

Assembly

- Place the electronic board inside the nebulizer_board_housing.
[The solder bridge was from the development phase and is not present in the final board.]

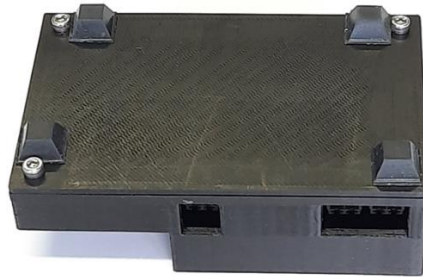


- Place the bottom plate onto the housing and drill (3 mm) marks through the three holes into the board.



Remove the board from the housing and drill (3.5 mm) three holes into the board, as shown in the image above.

- Insert the board again into the housing and mount the bottom plate with three M3x10 screws.
- Fix the four self-adhesive rubber feet onto the bottom plate.



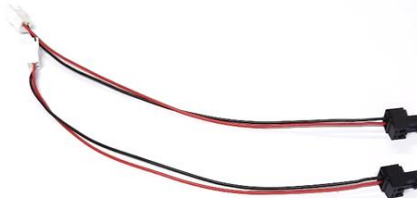
Ports for the atomizers

12 V port for the exhaust unit



12 V port for the power supply

- Connect each JST PH2 pin cable to a 2-position terminal block (polarity does not matter for the atomizers).



- Connect the rest of the cable assembly (2.5mm ID, 5.5mm OD, Plug to Wire Leads) to a 2-position terminal block (polarity important).



- Insert the terminal blocks into the respective ports of the board (see images above).
- Connect the 2.5 mm x 5.5 mm plug of the cable assembly to the exhaust unit.
- Connect the 2.5 mm x 5.5 mm plug of the 12 V power supply to the board.

Instruction S2 Adjusting the frequency for a different atomizer

- Open the file Firmware/src/main.cpp with visual Studio Code and scroll down to this section:

```
void setup(){
  noInterrupts();
  TCCR2A =
    1 << COM2A0 |
    1 << WGM21;
  TCCR2B =
    1 << CS20;
  DDRB =
    1 << DDB3; // PWM pin D11 (PB3) as output
  OCR2A = 73; //this value must be changed for a different atomizer's frequency
  interrupts();
}
```

Calculating the value for OCR2A

For example:

CPU frequency = **16000000** Hz (16 MHz), Arduino nano V3.x

atomizer frequency = 116 kHz

2 * atomizer frequency (toggled 50 %) = 232 kHz = **232000** Hz

$$OCR2A = \frac{16000000}{232000} - 1 = 67.96551724 \approx \mathbf{68} \text{ (must be integer)}$$

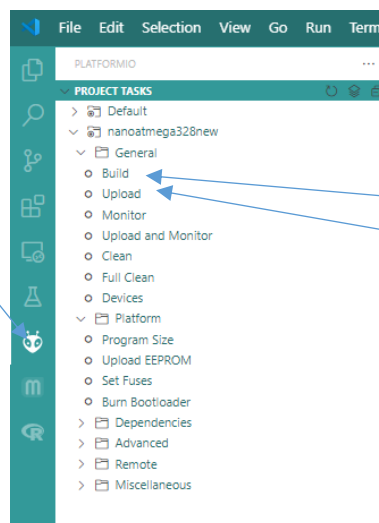
Check:

$$\text{adjusted atomizer frequency} = \frac{16000000}{68 + 1} = 231884.058 \text{ Hz} = 231.88406 \text{ kHz}$$

divided by 2 (50 %) => resulting frequency = 115.94203 kHz

- Exchange the value 73 by the new value 68.
- Save the file.

- Click the PIO button.
-



- Build the firmware.
- Upload the firmware.

Instruction S3 Operation and cleaning of the Nebulizer 20 cm x 10 cm

As precautionary advice, corrosive reagents, such as hydrochloric acid, should be substituted by other non-corrosive acids. Advantageously, the substitution of a damaged atomizer disc costs only about 2 Euros.

Operation

- Place the HPTLC plate under the cut-out in the Nebulizer casing.
- Connect the board to power.
- Turn the knob for intensity on the control unit counterclockwise until the display shows zero.
- Connect the atomizers to the JST PH2 pin cables.
- Fill in the same volume of reagent for nebulization into both atomizers.
- Turn the knob for intensity clockwise to reach the desired nebulization intensity to immediately start the nebulization (avoiding potential droplet formation).
- After the nebulization is finished, turn the knob for intensity again to zero.
- Let the fumes settle down for some minutes.
- Start the exhaust unit by pressing the knob for the pump on the control unit.
- Stop the exhaust unit by pressing the pump knob again.
- Take off the HPTLC plate.

Spraying takes typically 10 min.

If it takes longer, up to 30 min, some particles can have been formed (*e.g.*, solubility issue due to aggregation or solidification of molecules in the prepared solution) and may clog. You can try an atomizer disc with a wider pore size.

Several plates using the same solution or suspension can be sprayed in sequence without intermediate cleaning.

Cleaning

- Clean the atomizer holders by rinsing inside and outside with a suitable solvent (positioned over a waste beaker).
- A suitable rinsing solvent (1.5 mL; *e.g.*, water for aqueous solutions and suspensions) is nebulized onto paper towels.
- Set the intensity on the control unit to zero!
- Disconnect the atomizer holders, take them out of the Nebulizer and dry them inside and outside with a paper towel.
- Clean the Nebulizer parts with water and methanol or ethanol. Do not use acetone (destroys the PLA)!

Cleaning takes about 5 min.

It is a must, otherwise you may observe clogging for the next spraying process.