

Elveflow User Guide **MICROFLUDIDIC DROPLET PACK**

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Introduction



The droplet pack offered by Elveflow is designed for researchers with no prior knowledge in microfluidics. Simple and intuitive instructions are provided to quickly and easily make droplets and control droplet generation parameters.

When starting out, the user can follow step by step the provided protocol to obtain droplets of the specific size. In a second stage, the user can rely on the **numerous tips** provided and explore the **"going further"** section to complete its training in droplet generation and microfluidic flow control.

Two chips are provided with this pack allowing you to **create droplets within a diameter range from 10 to 80 µm.** The dimensions can be adapted to reach bigger or smaller sizes of droplets through various commercially available chips. In this droplet pack, the provided materials and experimental protocol are **fully versatile**. Thereby, it can be used with other types of microfluidic chips.

For more theory, you can refer to our whitepaper on droplet-based microfluidics

Droplet generation in a microfluidic chip

The two chips provided with the pack are based on **flow focusing droplet generator geometry.** Each chip is composed of four different nozzles providing a broad range of droplet sizes.

Through this geometry, **monodisperse droplets are formed by the combined action of the continuous and dispersed phases.** The dispersed phase (the phase that will become the inner phase of the droplets, here, the water) is squeezed between two flows of the continuous phase (the phase that will carry the droplets, here, the oil and surfactant), which leads to the formation of droplets. The surfactant contained within the continuous phase (e.g. the oil) stabilizes the oil/water interface, allowing the droplets to be stable in time and preventing droplet coalescence when in contact with each other.

A surfactant is an **amphiphilic molecule** that adsorbs at the oil-water interface, reduces the surface tension at the curved interface and stabilizes the resulting oil-water two-phase mixture. Thus, it allows the **suspension of water droplets in oil** (or Water-in-Oil emulsion) to be stable over time.

Both chips are made by injection molding in Topas polymer (COC) and are hydrophobic.

A hydrophobic surface ensures an effective water-in-oil droplet generation as the water droplet won't adhere to the channel walls.

Oil in water droplets can also be made with the same chips using a hydrophilic surface treatment to prevent oil droplets from adhering to the channel wall due to hydrophobic interactions, or with other chips (e.g. glass-based).

The **final features, properties and characteristics of the generated droplets** (size, frequency) depend on the chip geometry (shape and dimensions of the channels and the nozzle), the physical parameters of the liquid (surface tension, viscosities) and the channel wall surface treatment.





Droplet forming inside the microfluidic chip



Design and characteristics of microfluidic chips

Droplet size between [10 - 40] µm: Fluidic 947 from Microfluidic Chipshop

Interface type	Mini Luer
Nozzle sizes	10 -15 – 20 – 30 µm
Lid thickness	140 µm
Material	Topas

Droplet size between [50 - 80] µm: Fluidic 440 from Microfluidic Chipshop

Interface type	Mini Luer
Nozzle sizes	50 -60 – 70 – 80 μm
Lid thickness	140 µm
Material	Topas





For a given system (a microfluidic chip with defined continuous and dispersed phases), **the characteristics of the droplets generated will depend on the flow rates of the two immiscible phases.** The setup and experimental protocol given within this droplet pack will allow you to finely tune the flow rate of the two liquids to obtain the desired droplet properties.



Picture of the topas (COC) microfluidic chip

Contents of the microfluidic droplet pack

1 OB1 MK3+ Pressure-driven flow controller

2 channels from 0 to 2 bar

The pressure controller is the centerpiece of the setup, enabling accurate control over the pressure difference across the microfluidic system and thus, a fine control over the liquid flow in the microfluidic device.



PRINCIPLE OF PRESSURE-DRIVEN FLOW RATE CONTROL Elveflow instruments use pressure to drive liquid flows in fluidic systems The elementary principle is that a pressure difference across a fluidic line (between The OB1 pressure controller creates and controls the the outlet and the inlet of the fluidic system : ΔP = Pout-Pint) generates a motion pressure difference by pressurizing the air in a sealed of the liquid in the system characterized by its volumetric flow rate. The flow rate reservoir that contains the liquid at the inlet of the system. Q is proportional to the pressure difference, and the proportionality coefficient R is The outlet pressure Pout most often equals the atmospheric called the fluidic resistance: pressure, so the OB1 controls $\Delta {f P}$ $\Delta P = RxO$ R depends on the fluidic system geometrical characteristics and on the properties of the liquid. For instance: the longer or the thinner the tubing is or the more viscous the liquid is, the greater is the resistance. It means that a higher pressure difference is required to obtain a same flow rate in a system with longer or thinner tubing or with a more viscous liquid. viscous liquid. Viscosity Viscosity Length For instance the resistance of a circular cross channel is $R \propto \frac{\mu L}{r^*}$ radius Each element of the circuit (chip, tubing, sensors, ...) contributes to the whole resistance R of the system.

e OB1 pressure & 3. Pressurized older rack

A flow rate sensor can be added in line to measure and monitor the flow rate Q.

IN PRESSURE CONTROL: the user controls the pressure difference (the command is a pressure) and it generates a flow rate.

IN FLOW RATE CONTROL: the user sets the flow rate (the command is a flow rate) and the system continuously adapts the applied pressure difference so that the Q meets its targets.

You can refer to this video to learn more about pressure-driven flow control https://youtu.be/niWfINDUub4





2 Flow rate sensors #2 MFS-2-D

The standard mass flow sensor (MFS) measures the flow rate of the liquid going through the sensor. Combined with the pressure controller, it allows the user to monitor and precisely control the flow rate.

2 Topas (COC) microfluidic chips

The microfluidic chip is where the two immiscible phases (water and oil here) are precisely injected and mixed to generate monodisperse droplets (CV < 3%). The two microfluidic chips are made of Topas. Topas is a cyclic olefin copolymer (COC) resin which is a chemical relative of polyethylene and other polyolefin plastics. Both microfluidic chips contain 8 independent fluidic systems, and 4 different nozzles.

NB: Experimentations of this user guide have been conducted with Microfluidic ChipShop microchips.





1 bottle of surfactant in oil (FluoSurf 2% in HFE-7500, 10 mL)

FluoSurf (Emulseo) is a fluorinated surfactant specially designed and optimised to stabilise aqueous (water-in-oil) droplets in fluorinated oil (HFE 7500) and thus, to prevent droplet coalescence.

6 reservoirs and pressurized reservoir caps

Reservoirs are used to hold your liquids and are closed with specialized black reservoir caps that contain ports to connect the tubing. The pressure imposed by the OB1 pressure-driven flow controller in the reservoir forces the liquid to leave the reservoir through the pressurized cap and flow through the microfluidic system. Pressurized caps are autoclavable, infinitely reusable and are available for eppendorf tubes (1.5 mL) and falcon reservoirs (15 ml and 50 ml). Two of each type are included inside the droplet pack.





1 reservoir holder with 2 push-in connectors

A specific reservoir holder has been designed to securely hold your reservoirs and solutions.

It can hold up to two reservoirs with an easy-to-plug push-in connector ensuring a secure connection of your tubing to the reservoir in order to avoid any spill or leak from your system.

2 flow resistor systems

Flow resistors consist in the assembly of PEEK capillaries with small internal diameters. Those resistors are used to increase the resistivity of the microfluidic system to improve the stability and control of the flow rate in the system.

This Droplet Pack comes with 2 ready-to-use Flow Resistors, both allowing for operation over the full range of the pressure controller (0 to 2000 mbar):

- KFR-22-H for the dispersed phase. It enables stable control of flow rates ranging from 0,42 μ L/ min to 7 μ L/min using water or liquids with water-like viscosity (close to 1 mPa.s).
- KFR-22-I for the continuous phase. It enables stable control of flow rates ranging from 5 μ L/ min to 70 μ L/min for the case of oil (HFE-7500) or liquids with viscosities close to 1.24 mPa.s.



To estimate the microfluidic resistance in your system, you can refer to our online microfluidic calculator



1 Tubing 1/32" OD (30 m)

The liquid passes through the tubing to go from one component (such as reservoirs, MFS, chip...) to another. Its internal diameter is $300 \ \mu m$.

Other option: 1 Tubing Tygon 1/16" OD 500 μm ID" and a piece of silicone tubing from Microfluidic Chipshop (not included in this pack).

1 pack of Mini Luer connectors and plugs

The male Mini Luer fluid connectors are designed to connect the tubing to the Chipshop mini luer compatible microfluidic chips.

The male Mini Luer plugs are designed to block the unused ports of the Chipshop microchips.





Pneumatic tubing

To connect the OB1 outputs to the pressurized reservoirs.

1 Microfluidic connection kit

These pieces are required to connect the different components with the tubing.

Refer to appendix 1 for a visual guide to connections





1 air filter

This air filter is placed between the pressure generator (compressor for instance) and the ${\sf OB1}$ inlet.

It keeps the OB1 clean from dust and humidity.

1 Tubing cutter

This cutter is useful to properly cut the tubing (perpendicular and clean section), which helps to prevent leaks in your setup.





ESI software

The OB1 and the MFS are controlled by the software. Its interface allows you to easily select the pressure or the flow rate you want to set in the system.

The ESI software also contains a useful tool to automate your droplet generation: the ESI sequence scheduler. It allows you to define a set of actions and to connect them in sequence.

DOWNLOAD THE LATEST VERSION OF ESI SOFTWARE FOR FREE



This pack does not contain a pressure source.

The OB1 should be connected to a pressure source delivering a pressure between 2,5 and 10 bar. Refer to the OB1 user guide for more information.

Before starting the experiment



An OB1 calibration is required for the first use. Once calibrated, it is not necessary to do so before each experiment, unless it drifts slightly or if you change the pressure source. Once it is calibrated, you can connect it to any other component of the system to perform experiments.

!

Don't forget to **set up the provided filter** between **the pressure source** and the **OB1**.

Refer to **appendix 1** to see how to connect all the components together



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Y IS IT NECESSARY TO MFS ?	f a liquid by locally warming it a lifterent locations of the sensor	nd by capillary.	ESI - 3.05.00 – • × C HELP US P E C K TO IMPROVE C C K III O OB1 DROPLET_GENERATION
elation between temperature measure hysical properties of the liquid passing uired. Two calibrations are implemen	ement and the flow rate highly o g through the MFS. That is why ted directly in the MFS : Water a	depends on a calibration and Isopropyl.	
/ater calibration is suited for all aque ropriate for all the carbon chains (fluc ment.	ous solutions. The calibration Iso prinated oil), with an additional I	opropyl inear	
ELVEL FLOW (in out) se	ith the MFS-2-D Oil : it is ne use a scale factor and an off tting the calibration to Isopr	cessary fset after opyl .	ADD INSTRUMENT FlowSensor OIL FlowSensor WATER
OIL_FlowSensor (Sensor) Configuration General Sensor info Connected to Scaling	Name OIL Type Digital Flow Sensor	CIL FlowSensor	ADD SENSOR
		General Sensor info Connected to Scaling	Choose Resolution
Detering an instrument the database. You can no * Modifications in fields marke	or sensor erases all its information from ead it as new from the main menu.	Delete e is relaunched.	Offset 0 Parameter to measure Volumetric Flow rate
Based on our experience with use the following values of o MFS2 measuring oil flow rat	HFE7500, we advise you to ffset and scale factor for the es:		* Modifications in fields marked with an asterisk will be applied once the software is relaunched.
SCALE FACTOR	OFFSET]	
7,24	0		I NEED TO RECALIBRATE MY MICROFLUIDIC FLOW SENSOR https://support.elveflow.com/support/solutions/ articles/48001163077
For other oils, please proceed The MFS-2-D with the calibra	to the calibration following ation Water can measure flo	the user guide ww rates between 0 ,4	l2 and 7 μl/min.
The MFS-2-D with the calibra	ation Isopropyl can measure	oil flow rates betwe	en 5 and 70 μl/min.

Let's start making droplets!

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Let's start making droplets!

Set up the reagents

Water phase: Attach the reservoir (1,5, 15 or 50 ml) filled with water to the pressurized reservoir cap and connect it to the supplied 1/32" OD tubing and fittings.

Oil phase: Attach the reservoir (1,5, 15 or 50 ml) filled with HFE-7500 oil + 2% Fluosurf surfactant to the pressurized cap and connect it to the supplied 1/32" OD tubing and fittings.



Refer to appendix 1 to see how to connect all the components together

Plug both reservoirs to the reservoir holder and to the corresponding OB1 pressure controller outlet.



Elveflow Microfluidic Reservoirs Assembly Instructions https://support.elveflow.com/support/solutions/articles/48000983958-ob1-setupinstallation-instructions

σP

The percentage of surfactant required will depend on your experiment (stability and integrity of your droplets). For typical water-in-oil droplets, 0.5% to 2% of Fluosurf surfactant is enough to generate, collect and reinject the droplets.



All the liquid must be preferably filtered and not exposed to the environment, as dust will settle in it. It is recommended to manipulate and put the caps inside a fume hood that avoids airborne particles settlement.

2

Set up the microfluidic chip



Add **Mini Luer connectors to the inlets and outlets of the microfluidic chips** for the fluidic system chosen. Add plugs to inlets and outlets of the other devices to avoid dust settling inside.

JU

The use of thread seal tape is essential to avoid leaking from the connectors of the chips. However, only one layer should be applied or else it can end up breaking the interface on the microfluidic chip. This layer must be changed after each use and, potentially, if leakage is observed.

JD

If water is attaching to the walls of your channel, treat the microfluidic chip with a hydrophobic treatment, (Aquapel from Autoserv, or Rain X (ITW Glodal Brands). Treatment should preferably be done inside a fume hood to prevent dust, particles or fibers from entering in the microfluidic chip. The chip must be dry while inserting the liquid, left to rest 3-5 min minutes, and rinsed with water or oil. We recommend repeating the process 3 times. After rinsing it is important to dry the chip by air injection (unless you are using Aquapel).

Set up the microfluidic system

Set up the following system.

Channel 1

2000 mbar

The MFS-2-D Water is connected between the **water reservoir** and the **flow resistor KFR-22-H** using the supplied 1/32" OD tubing and fittings. The resistance is connected to the microfluidic chip inlet for the continuous phase (first inlet).

Channel 2

2000 mbar

The MFS-2-D Oil is connected between the **oil reservoir** and **the flow resistor KFR-22-I** using the supplied 1/32" OD tubing and fittings. The resistance is connected to the microfluidic chip inlet for the dispersed phase (second inlet).

Refer to **appendix 1** to see how to connect all the components together

WHY IS IT IMPORTANT TO WORK WITH RESISTANCES?

Adding the right amount of fluidic resistance in the fluidic path is important as it is the way to finetune the system's whole resistance R to obtain the best performance in terms of flow rate control. It allows to adjust R so that the range of accessible ΔP (given by the range of the OB1 channel output) matches the range of the flow rate sensor used. Doing so, the system uses the whole dynamic range of both the sensor and the regulator to control the flow rate.

For instance in a system (with a 2 bar OB1 channel) where R=R1 is too low, few mbar of ΔP generate a high flow rate that can saturate the flow rate sensor.

In other terms, a small part of the accessible ΔP matches the full range of the sensor, it can lead to unstable flow control: a small ΔP variation triggers a huge flow rate variation. The solution is to add the right amount of fluidic resistance to increase R=R2 so that most of the range of the pressure regulator is used: a higher pressure is required to saturate the sensor

and consequently a small ΔP variation

triggers a small flow rate variation, it allows to obtain a stable system.

YOU'RE NOT FAR FROM YOUR FIRST DROPLETS!

Fill the microfluidic chip

Make sure your reservoirs are filled with their respective liquids. Turn on the OB1 and launch the ESI software.

Open the OB1 window in the ESI.

Set the oil channel (here, channel 2) pressure to **100 mbar** until the chip is filled (it should take less than one minute). Once the microfluidic chip is filled, decrease the pressure of the oil channel to **50 mbar**.

You can see that the chip is filled when the oil is going through the outlet after having passed through the chip (a difference of color within the channel of the microfluidic chip can be observed when the oil flows through it).

Then, **set the water channel** (here, channel 1) pressure to **100 mbar** and slowly increase the pressure until you

see both phases in the microfluidic chip and droplets are starting to be generated (aqueous phase overcoming the back pressure of the oil phase).

You might see **air bubbles** after having set the water channel pressure, before the water has reached the chip. Wait for the air in the system to be completely replaced by water. Air bubbles tend to be more contrasted than water droplets.

Refer to **Troubleshooting** if this does not work

JJU

To reduce the formation of bubbles and shorten the initial filling time, it can be useful to fill the tubing from the reservoir to the chip with liquid before connecting the chip. Set a low mbar command on the OB1 and watch the liquid interface move through the tubing. Set the pressure to zero when the interface reaches the tubing extremity then connect the extremity to the chip. 9

Make sure that the outlet reservoir is not totally empty, and that the outlet tubing plunges into the solution, otherwise there will be dripping, which will perturb the stability of the flow rate.

Congratulations, you now control the pressure. This is enough to make droplets! But if you want to control the flow rate and thus have more precise control over the size and frequency of the droplets, there are a few last things to set up.

) WHY CONTROL THE FLOW RATE RATHER THAN THE PRESSURE?

The characteristics of your droplets are determined by the flow rates of both dispersed and continuous phases. Requesting a flow rate instead of imposing a pressure has mainly two advantages:

- The flow rate stays constant throughout time, even though the experimental conditions slightly change (if a bubble or clogging occurs, the system tries to compensate and to maintain a constant flow rate.)
- It compensates for the hydrostatic pressure changes. The inlet pressure of the fluidic system is equal to the atmospheric pressure, plus the pressure imposed by the OB1 on the air above the liquid in the seal reservoir, plus the pressure exerted by the water column (height of liquid between the water/air interface in the reservoir and the tube inlet at the bottom of the reservoir) around 1 mbar per cm of water. So when the liquid level decreases, the ΔP decreases over time so will Q in pressure control. In Flow rate control the ΔP is adjusted to maintain a constant flow rate.
- Even if your setup changes from one experiment to another, the same flow rates will lead to the same droplets (whereas when changing your setup, the fluidic resistances will change too and the same pressure will lead to a different flow rate).

Control of the flow rate

The last step is to set a flow rate feedback loop.

The feedback loop consists of a constant adaptation of the OB1 pressure output to impose a flow rate in the system as close as possible to the flow rate targeted by the user. The feedback loop relies on an algorithm (PI Basic) which depends on two parameters : P and I. The user can set these parameters to match the requirements in terms of responsiveness and stability.

REFER TO AN APPLICATION NOTE

To see all the details https://support.elveflow.com/support/solutions/ articles/48001142611-set-a-flow-control-feedback loop-resistance-and-pid-tuning-

To have very monodisperse droplets, Elveflow advises to set low values of P and I, which will decrease the responsiveness of the system but guarantee its stability. Elveflow advises you to begin with the following feedback parameter values:

SENSOR TYPE	р	I
MFS-2-D measuring oil flow rate	0,01	0,005
MFS-2-D measuring water flow rate	0,01	0,005

These values are appropriate for this setup and for all pairs of water and oil flow rates, and give very stable flow rates.

However, be aware that the responsiveness of the system with these parameters is quite low: up to 3 minutes may be needed before the flow rates stabilize (especially when working with low flow rates such as 2μ /min for the water).

If you have another setup (especially, if you use different flow resistor systems) and/or depending on your needs (if you want to increase the responsiveness for instance), you can fine-tune the values of the feedback parameters.

To learn more about the tuning of the P and I parameters, please refer to the going further section.

DROPLET_GENERATION Several algorithms are available. ELVE PLUG & PLAY ~ Elveflow advises to use the "PI Basic" algorithm. DROPLET_GENERATION Ch.2 cfg DROPLET GENERATION Ch 2 cfc îţî îŝî Π 1 1 4 $\Delta \Delta$ л . A. Max, Press, Slope [bar/s] <u>^</u> 1. o Tun 500.00 Reg. Max. Sens. Max > 10.00 10.00 0.00 0.00 2000.00 70.00 Reg. Min Sens. Min 0.00 -70.00 Click and type the values of P and I, it will be easier

To set the feedback parameters, follow these instructions:

Now, you can start the feedback loop of flow rate control by **choosing the sensor control mode** (flow rate control instead of pressure control), and fixing the flow rate you want to have (you are now controlling the flow rate instead of the pressure):

!

Don't forget to make sure that the whole fluidic system is filled with liquid before switching to flow rate control mode.

For example, you can set the water flow rate to **1 µL/min** and the oil flow rate to **50 µL/min** to make your first droplets. The dependency of droplet size and frequency on oil and water flow rates is given in the following section.

ΤP

While the tubing is filled with air, you should not control the flow rate but the pressure. Indeed, the MFS filled with air will not measure any flow rate, and the OB1 will keep increasing the pressure until the fluid finally reaches the MFS or the pressure reaches its maximum, which should be avoided.

Once the fluid has reached the MFS, it is safe to switch to flow rate control.

τP

Increasing the value of the parameter I in the PID Basic algorithm while controlling the flow rate ("sensor" mode) will induce an overflow. If you want to prevent this overflow, only change the values of the parameter I while controlling the pressure ("Regulator" mode) then switch back to flow rate control.

Characterisation of the droplets

Generally, increasing the oil flow rate will result in increasing the frequency of production of the droplets.

Droplet size and production frequency can be fine tuned depending on the flow rates of the dispersed and continuous phases and the size of nozzle used, as shown in the tables below.

Fluidic 440 _ Droplet Generator Chips _ Multi Channel Design

Design # 1 and # 2: 80 μ m nozzle (CV < 2%)

UL/min OIL WATER	50	52	54	56	58	60	62	64	66	68	70
0.4	83 μm (13 Hz)	83 μm (14 Hz)	83 μm (14 Hz)	82 μm (15 Hz)	82 μm (15 Hz)	82 μm (16 Hz)	81 µm (18 Hz)	80 µm (19 Hz)	80 µm (19 Hz)	79 μm (20 Hz)	79 μm (20 Hz)
0.6	> 80 µm	83 μm (26 Hz)	82 µm (26 Hz)	82 µm (29 Hz)	82 µm (30 Hz)	81 µm (31 Hz)	81 µm (32 Hz)	81 μm (34 Hz)	81 µm (34 Hz)	80 µm (36 Hz)	80 µm (36 Hz)
0.8	> 80 µm	> 80 µm	> 80 µm	> 80 µm	83 μm (45Hz)	83 μm (45 Hz)	82 μm (46 Hz)	82 μm (49 Hz)	82 μm (49 Hz)	81 µm (51 Hz)	81 μm (51 Hz)
1	> 80 µm	83 μm (47 Hz)	83 μm (49 Hz)	83 μm (52 Hz)	83 μm (54 Hz)	82 μm (57 Hz)	82 μm (57 Hz)				

Design # 3 and # 4: 70 μ m nozzle (CV < 2%)

UL/min OIL WATER	52	54	56	58	60	62	64	66	68	70
0.6	73 μm (43 Hz)	72 μm (45 Hz)	71 μm (46 Hz)	71 µm 47 Hz)	70 μm (48 Hz)	70 μm (48 Hz)	70 μm (50 Hz)	69 μm (51 Hz)	69 μm (52 Hz)	68 μm (53 Hz)
0.8	>70 µm	>70 µm	73 µm (56 Hz)	72 µm (58 Hz)	71 µm (59 Hz)	71 µm (61 Hz)	70 µm (62 Hz)	70 µm (63 Hz)	69 µm (64 Hz)	69 μm (67 Hz)
1	>70 µm	>70 µm	73 µm (67 Hz)	73 µm (68 Hz)	72 µm (69 Hz)	72 μm (71 Hz)	71 μm (72 Hz)	70 µm (74 Hz)	70 µm (76 Hz)	70 μm (77 Hz)
1.2	>70 µm	>70 µm	>70 µm	73 µm (79 Hz)	72 µm (81 Hz)	72 μm (83 Hz)	71 µm (85 Hz)	71 µm (88 Hz)	71 µm (90 Hz)	70 μm (93 Hz)
1.4	>70 µm	>70 µm	>70 µm	73 μm (79 Hz)	73 μm (95 Hz)	72 μm (81 Hz)	72 μm (101 Hz)	71 µm (101 Hz)	71 µm (104 Hz)	71 μm (107 Hz)
1.6	>70 µm	>70 µm	>70 µm	>70 µm	73 µm (107 Hz)	73 μm (111 Hz)	72 µm (115 Hz)	72 μm (119 Hz)	72 μm (123 Hz)	71 μm (128 Hz)
1.8	>70 µm	73 μm (123 Hz)	73 μm (128 Hz)	72 μm (133 Hz)	72 μm (139 Hz)	71 μm (145 Hz)				

Design # 5 and # 6: 60 μ m nozzle (CV < 2%)

ul/min OIL WATER	50	52	54	56	58	60	62	64	66	68	70
0.5	62 μm (69 Hz)	61 µm (71 Hz)	61 μm (72 Hz)	60 µm (74 Hz)	59 µm (83 Hz)	57 μm (85 Hz)	58 μm (88 Hz)	58 µm 90 Hz)	57 μm (95 Hz)	57 μm (98 Hz)	56 μm (101 Hz)
1	> 60 µm	62 μm (95 Hz)	62 μm (98 Hz)	61 µm (101 Hz)	61 µm (104 Hz)	60 µm (107 Hz)	60 µm (111 Hz)	59 µm (115 Hz)	59 µm (119 Hz)	58 µm (123 Hz)	57 μm (128 Hz)
1.5	> 60 µm	> 60 µm	62 μm (133 Hz)	61 µm (139 Hz)	61 µm (145 Hz)	60 µm (151 Hz)	60 µm (159 Hz)	59 µm (167 Hz)	58 μm (175 Hz)	58 µm (185 Hz)	58 μm (196 Hz)
2	> 60 µm	> 60 µm	> 60 µm	62 μm (151 Hz)	62 μm (159 Hz)	61 µm (167 Hz)	60 µm (175 Hz)	60 µm (185 Hz)	59 μm (196 Hz)	59 µm (208 Hz)	59 μm (222 Hz)
2.5	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 μm (167 Hz)	62 μm (175 Hz)	61 µm (185 Hz)	61 µm (196 Hz)	60 µm (222 Hz)	60 µm (238 Hz)	59 μm (256 Hz)
3	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 μm (208 Hz)	62 μm (217 Hz)	61 µm (238 Hz)	61 µm (250 Hz)	60 µm (263 Hz)	60 μ (278 Hz)
3.5	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 μm (250 Hz)	61 µm (263 Hz)	61 µm (278 Hz)	60 µm (294 Hz)	60 µm (312 Hz)
4	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	> 60 µm	62 μm (278 Hz)	61 µm (294 Hz)	60 µm (312 Hz)	60 µm (333 Hz)

Design # 7 and # 8: 50 μm nozzle (CV < 2%)

µL/min OIL WATER	50	52	54	56	58	60	62	64	66	68	70
0.6	> 50 µm	> 50 µm	> 50 µm	> 50 µm	50 µm (75 Hz)	49 μm (77 Hz)	49 μm (79 Hz)	48 μm (80 Hz)	48 μm (82 Hz)	47 μm (84 Hz)	47 μm (85 Hz)
1	52 µm (81 Hz)	51 µm (82 Hz)	51 µm (85 Hz)	50 µm (86 Hz)	50 µm (87 Hz)	49 μm (90 Hz)	49 µm (92 Hz)	49 µm (93 Hz)	48 µm (97 Hz)	48 µm (98 Hz)	47 μm (102 Hz)
1.4	52 µm (93 Hz)	52 µm (95 Hz)	51 µm (98 Hz)	51 µm (100 Hz)	50 µm (102 Hz)	50 µm (106 Hz)	49 µm (108 Hz)	49 µm (112 Hz)	49 μm (117 Hz)	48 µm (120 Hz)	48 μm (122 Hz)
1.8	> 50 µm	52 µm (108 Hz)	52 µm (110 Hz)	51 µm (115 Hz)	51 µm (117 Hz)	50 µm (120 Hz)	50 µm (122 Hz)	49 µm (128 Hz)	49 µm (131 Hz)	48 µm (134 Hz)	48 μm (138 Hz)
2.2	> 50 µm	> 50 µm	52 µm (125 Hz)	52 µm (128 Hz)	51 µm (131 Hz)	51 µm (138 Hz)	50 µm (141 Hz)	50 µm (149 Hz)	49 µm (153 Hz)	49 µm (157 Hz)	48 μm (162 Hz)
2.6	> 50 µm	> 50 µm	> 50 µm	52 μm (145 Hz)	51 µm (153 Hz)	51 µm (157 Hz)	50 µm (162 Hz)	50 µm (167 Hz)	49 µm (172 Hz)	49 µm (184 Hz)	49 μm (190 Hz)
3	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (178 Hz)	51 µm (184 Hz)	51 µm (190 Hz)	50 µm (197 Hz)	50 µm (204 Hz)	49 µm (212 Hz)	49 μm (220 Hz)
3.4	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (197 Hz)	51 µm (204 Hz)	51 µm (212 Hz)	50 µm (220 Hz)	50 µm (230 Hz)	49 μm (240 Hz)
3.8	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (220 Hz)	51 µm (230 Hz)	51 µm (240 Hz)	50 µm (251 Hz)	50 µm (262 Hz)
4.2	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (251 Hz)	51 µm (262 Hz)	51 µm (276 Hz)	50 µm (290 Hz)
4.6	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 µm (290 Hz)	51 µm (306 Hz)	51 μm (324 Hz)
5	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	> 50 µm	52 μm (344 Hz)	51 µm (367 Hz)

Fluidic 947 _ Droplet Generator Chips _ Multi Channel Design

Design # 7 and # 8: 30 μm nozzle (CV < 1.5 %)

WATE	OIL R	50	54	60	63	67	70
0).5	30 µm (109 Hz)	< 30 µm				
	1	< 30 µm	30 µm (163 Hz)	< 30 µm	< 30 µm	< 30 µm	< 30 µm
1	.5	> 30 µm	< 30 µm	30 µm (235 Hz)	< 30 µm	< 30 µm	< 30 µm
	2	> 30 µm	< 30 µm	> 30 µm	30 µm (294 Hz)	< 30 µm	< 30 µm
2	2.5	> 30 µm	> 30 µm	< 30 µm	< 30 µm	30 µm (385 Hz)	< 30 µm
	3	< 30 µm	30 µm (417 Hz)				

Design # 5 and # 6: 20 μ m nozzle (CV < 1.5 %)

Design # 3 and # 4: 15 μ m nozzle (CV < 2 %)

μι Wate	/min OIL R	60	70
().5	14.5 µm (590 Hz)	polydisperse
).8	polydisperse	14.9 μm (752 Hz)

```
Design # 1 and # 2: 10 µm nozzle (CV < 3%)
```


UL/min OIL WATER	60	70
0.5	9.5 µm (910 Hz)	polydisperse
1	polydisperse	9.7 μm (1270 Hz)

After generating your droplets

5

Once your experiment is finished, we recommend that you fill the channel with oil to protect the chip from all contaminants that could settle inside or damage the channels, before disconnecting the tubings, and storing the microfluidic chip in a box or covering interfaces with tape.

Make sure to empty the tubings and to properly clean the flow rate sensors according to the cleaning procedures described in the Flow Rate Sensor user guides and in the support section of the Elveflow website.

Switch off the OB1 pressure controller and the pressure supply inlet.

All the droplets pictures were taken with the FASTEC IL5 camera

Troubleshooting & Going further section

Troubleshooting

The flow rate measured by my MFS suddenly drops down for a short duration

The reason is probably that you have air bubbles in your system. When an air bubble passes through the MFS, the sensor measures a zero flow rate as long as the bubble is inside. To get rid of air bubbles, just wait for it to get out by maintaining a constant pressure or flow rate in both channels. The initial filling of the system is critical to avoid bubbles.

The flow rate doesn't stabilize

If the flow rate can't stabilize itself on the fixed value, first check that you haven't forgotten to put the flow resistor in the fluidic system. If it's not the cause of the problem, you should consider changing the values of P and I.

The lower the values of P, the more the flow rate will be stable. The **"going further"** section details this solution.

Another way of solving this problem is to increase the microfluidic resistance. The **"going further"** section details this solution.

I see only one phase (water or oil) in my chip

Check both your MFS. The MFS should be connected in the right direction (indication of inlet/outlet are depicted on top of the sensor). If both MFS are connected in the right direction and are measuring a positive flow rate, and if the situation is lasting too long, check all your connections, there must be a leak.

If one of the values measured by the MFS is negative:

- if you are controlling the pressure, increase the pressure of the channel flowing in the bad direction
- if you are controlling the flow rate, the flow rate should gradually increase to the fixed value. If it takes too much time, you can open the flow control configuration of the channel flowing in the wrong way, and gradually increase the value of I in the PI Basic

algorithm (refer **3** Control of the flow rate **p.13**

I have dust in my chip/my chip is clogged

In case your microfluidic chip starts to clog (dust, particles, etc.), try to increase the pressure (or flow rate) of both phases to expel the dust from the chip. If it doesn't work, you should change the device you are working with. The presence of dust in the chip should not be ignored. The monodispersity of the droplets can't be ensured in this case, and the size of the droplets could significantly change from the expectations of the user.

Working with "clean" solutions and reservoirs is essential to prevent chip clogging. We highly advise to filter solutions before using them in a microfluidic chip.

My aqueous phase sticks to the wall downstream of the generation region

As the hydrophobicity from the surface can sometimes wear off during the experiment, the aqueous phase can create a fluid path and prevent

droplet generation. If that happens, it is best to reduce the water flow rate so that the aqueous phase recedes to a position upstream of the droplet generation region. From there, slowly increase the flow rate to the desired value again to continue producing droplets.

My OB1 is very noisy, what is the problem?

If your OB1 starts making loud noise, it's probably because of a leak in your system: the pressure source tries to permanently compensate for the lack of pressure due to the leak.

Check all the connections of the system (using teflon tape is often useful to avoid leaks on fluidic and pneumatic connections).

I don't have the same results as those provided in the diagram

If your droplets don't have the size you expected, the reason could be a light systematic error of the MFS or the chip dimensions. You should consider calibrating the MFS once again or adjusting the flow rate values to get the target droplet size

This PDF is dedicated to the problem https://support.elveflow.com/support/solutions/articles/48001163077

Be careful when controlling very low oil flow rate (close to 5 μ l/min) with the MFS-2-D, even though the measurements of the MFS are highly repeatable, they could lack accuracy in this range of flow rates.

Refer to the MFS user guide for more details

I would like to produce droplets with a lower frequency, is it possible with this chip?

It is possible, but you will have to reach very low water and oil flow rates that the MFS can precisely measure. It is possible to reach these low flow rates thanks to the control of the pressure with the OB1, but the stability of the flow rates (and thus the monodispersity of the droplets) could not be as good as with the control of the flow rates.

I would like to make oil in water droplets, can I do it just by switching the water and the oil inlet?

To generate these droplets, an hydrophilic chip should be used (e.g a glass chip). Please feel free to reach out to our technical sales team **contact@elveflow.com** for more information about it.

Going further

The droplet pack is very flexible. Depending on your needs, you could find it beneficial to change elements of the setup: for example, working with another chip, flow resistor system, or even other liquids.

You must be aware that, even though it is not hard to adapt your setup to new experimental conditions, there always are a few elements to adjust.

This section is here to introduce further upgrades and improvements to the system to perfectly taylor it to your specific experiment!

TP

How to make other droplets than those given in the diagram

Diagrams in <u>4</u> Characterisation of the droplets p.15 show you the monodisperse droplets you can do with the microfluidic chips supplied in the droplet pack. If your application requires droplets with other characteristics (e.g. size, throughput) than the one provided in this pack, other commercial microfluidic chips are available.

If you want to make oil in water droplets, you will need a chip made of hydrophilic material (such as glass or with appropriate treatment).

Calibrate the flow rate sensor if you are using a different liquid

If you plan to use an alternative oil you will have to calibrate the flow rate sensor, following the calibration procedure.

Calibration procedure
https://www.elveflow.com/microfluidic-applications/setup-microfluidic-flow-
control/microfluidic-flow-restrictors/

Adapt the fluidic system

By changing your microfluidic setup (e.g. microfluidic chips, liquid viscosity), the total fluidic resistance of the setup will be different.

Therefore, an adaptation of the flow resistor system and potentially the flow sensor is needed to allow the range of the pressure controller to match the range of the flow rate used.

To learn more about the resistances https://www.elveflow.com/microfluidic-applications/setup-microfluidic-flow control/microfluidic-flow-restrictors/

To learn more about Flow Resistance

https://support.elveflow.com/support/solutions/articles/48001142611-set-a-flow-control-feedback-loop

Change the values of P and I in the PI Basic algorithm

Whenever your setup changes (new liquids, new chip...), you will have to check that your feedback parameters **P** and **I** are still appropriate. Even if you keep the same setup, you could find benefit in changing the values of **P** and **I**.

The values of \mathbf{P} and \mathbf{I} Elveflow advises to set for the PI Basic algorithm are adapted for all couples of water and oil flow rates for this particular chip. Nevertheless, they can't be optimized for all couples.

If you want to make droplets of a specific size and you know the flow rates you will need, you can gradually change the values of \mathbf{P} and \mathbf{I} until you find more optimized ones for for these flow rates (more responsiveness or more stability).

To learn where and how to change and choose the values of P and I

4

in the PI algorithm, refer to

Characterisation of the droplets **p.15**

To learn more about the tuning of the feedback parameters https://www.elveflow.com/ /support/solutions/articles/48001142611-set-aflow-control-feedback-loop-resistance-and- -tuning

Be careful when changing the values of the parameter I. Suddenly increasing the value of the parameter I of the PI Basic algorithm will induce an overflow. You can avoid this overflow by changing the value of I while controlling the pressure instead of the flow rate.

Generally speaking, the higher the value of I, the more responsive the system will be, but the the less stable it will be once it has reached the requested value of the flow rate. If the value of I is too high, the flow rate will not stabilize.

If when requesting a low flow rate (for example, 1 $\mu l/min$ for the water) the system is really too slow to stabilize, carefully increase the value of I.

Increasing the value of ${\bf P}$ could increase the responsiveness of the system, and sometimes reduce the oscillation of the flow rate, but if it is too high, the flow rate will not stabilize.

As it can be seen on the diagrams in the "Characterisation of the droplets" part, the parameters of the oil-to-water flow rate ratios and throughput to generate droplets between 10 and 15 µm are not optimal, using Fluidic 947 microfluidic chip.

A simple change of configuration within the microfluidic setup can be done to improve those actions, by a change of the flow sensor and therefore a change of the flow resistor system. By changing the MFS2 for a MFS3 flow sensor for the oil phase, a higher flow range of up to 500 μ L/min can be achieved and therefore, a higher versatility of oil-to-water flow rate ratios can be reached.

1 Flow rate sensor #2 MFS-2-D 1 Flow rate sensor #3 MFS-3-D

The standard mass flow sensor (MFS) measures the flow rate of the liquid going through the sensor. Used with the pressure controller, it allows the user to monitor and precisely control the flow rate.

2 flow resistor systems

Flow resistors consist in the assembly of PEEK capillaries with small internal diameters. Those resistors are used to increase the resistivity of the microfluidic system to improve the stability and control of the flow rate in the system.

This Droplet Pack comes with 2 ready-to-use Flow Resistors, both allowing for operation over the full range of the pressure controller (0 to 2000 mbar):

- KFR-22-H for the dispersed phase. It enables stable control of flow rates ranging from 0,42 μL/min to 7 μL/min using water or liquids with water-like viscosity (close to 1 mPa.s).
- KFR-23-I for the continuous phase. It enables stable control of flow rates ranging from 25 μ L/min to 500 μ L/min for the case of oil (HFE-7500) or liquids with viscosities close to 1.24 mPa.s.

9	ELVE) FLOW	

μl/min OIL WATER	65	70	80	85	90
0.5	10 µm (910 Hz)	< 10 µm	< 10 µm	< 10 µm	< 10 µm
1	> 10 µm	10 µm (1270 Hz)	< 10 µm	< 10 µm	< 10 µm
1.5	> 10 µm	> 10 µm	10 µm (1830 Hz)	< 10 µm	< 10 µm
2	< 10 µm	> 10 µm	> 10 µm	10µm (2120 Hz)	< 10 µm
2.5	< 10 µm	< 10 µm	> 10 µm	> 10 µm	10 µm (2520 Hz)

FLUIDIC 947 _ Droplet Generator Chips: 10 µm nozzle (CV < 3%)

FLUIDIC 947 _ Droplet Generator Chips: 15 µm nozzle (CV < 2%);

UL/min OIL WATER	60	70	90	105
0.5	15 µm (590 Hz)	< 15 µm	< 15 µm	< 15 µm
1	> 15 µm	15 μm (830 Hz)	< 15 µm	< 15 µm
1.5	> 15 µm	> 15 µm	15 μm (1310 Hz)	< 15 µm
2	> 15 µm	> 15 µm	> 15 µm	15 μm (1970 Hz)

Appendix A All the possible connections

Connect the OB1 to the pressure source

Connect your OB1 pressure controller to external pressure supply using pneumatic tubing.

REFER TO AN OTHER USER GUIDE

Refer to the OB1 user guide for detailed instructions

Appendix A

Appendix A

Appendix B Detail of microfluidic chips

Supplementary information

Conditions of use

Terms and conditions of use

We strongly believe in the intrinsic quality of our microfluidic instruments line and we hope that you will be pleased with your purchase. However, in the unlikely event that you should receive damaged or incorrect goods in your delivery, please notify us within 7 days.

You will be offered the option of a refund or an exchange (provided the goods are in stock).

You may be asked to return goods for inspection. In this case we will refund the shipping fees.

Should the damaged or incorrect item be no longer available, you will be given the option of a refund. Please note that goods that become damaged or broken after 7 days of receipt cannot be returned.

Unwanted items

If for any reason you do not wish to keep your purchase and would like a store credit, then please notify us within 7 days.

We cannot accept unwanted returns that have been opened, used or damaged by the customer.

For unwanted goods, we allow up to 14 days for the return of goods. We will only issue a credit

upon receipt of all returned goods.

Please note that we are unable to refund your costs in returning unwanted goods or the delivery costs of sending the goods to you in the first place.

Cancellations

If you wish to cancel your order please email us immediately: contact@elveflow.com.

Privacy Policy

Customer details remain private and confidential and will not be released to a third party unless required to do so by law.

We use the information we collect about you to process orders, to provide a more personalized shopping experience and, if you request it, to notify you about new products, special offers or other information that may be of interest to you. We do not sell or pass on any personal information to any other Companies or Organizations.

Payments & Procedures

Payment from private customers must be paid upfront. Trade orders from registered companies or organizations can be invoiced. Payment is due strictly within 30 days of the invoice date.

Products & Prices

Please note that some goods may vary in style, color or detail from the image shown. We reserve the right to change prices at any time.

Transport and storage

Be careful not to harm or shake Elveflow[®] products while moving. Elveflow[®] products must not be transported when plugged in. Store products in standard conditions in an adapted box (typically the one used to send you the product).

Humidity and temperature must not exceed those of the specifications.

Exclusive remedies

The remedies provided herein are the customer's sole and exclusive remedies. Elveflow® shall not be liable for any direct, indirect, special, incidental, or consequential damages, whether based on contract, tort, or any other legal theory.

Safety Information

THE FOLLOWING GENERAL SAFETY PRECAUTIONS MUST BE FOLLOWED DURING ALL PHASES OF OPERATION, SERVICE, AND REPAIR OF THIS INSTRUMENT. FAILURE TO COMPLY WITH THESE PRECAUTIONS OR WITH SPECIFIC WARNINGS ELSEWHERE IN THIS MANUAL VIOLATES SAFETY STANDARDS OF DESIGN, MANUFACTURE, AND INTENDED USE OF THE INSTRUMENT. ELVESYS ASSUMES NO LIABILITY FOR THE CUSTOMER'S FAILURE TO COMPLY WITH THESE REQUIREMENTS.

Important advice

Pressurized Equipment

Elveflow[®] products are for research use only.

No liquid should get into the OB1, otherwise this would void the warranty.

The pressure source connected to the OB1 must be dry, dust and oil free, and of a maximum of 10 bar. Please take the required action to ensure that these conditions are met and maintained.

Conditions of use

This instrument is intended for indoor use. It is designed to operate at a maximum relative humidity of 60% and at altitudes of up to 2000 meters. Operating temperature range is +5 °C to 50 °C.

Do not operate in wet/damp conditions: to avoid electric shock, do not operate this product in wet or damp conditions.

Do not operate in an explosive environment. Do not operate the equipment in the presence of explosive or flammable gases or fumes.

Warning: Do not use this product as safety or emergency stop devices or in any other application where failure of the product could result in personal injury. The protective features of this product may be impaired if it is used in a manner not specified in the operating instructions. Before installing, handling, using or servicing this product, please consult the data sheet and user manual.

Failure to comply with these instructions could result in death or serious injury. If the buyer purchases or uses Elveflow® products for any unintended or unauthorized application, the buyer shall defend, indemnify and hold harmless Elveflow® and its officers, employees, subsidiaries, affiliates and distributors against all claims, costs, damages and expenses, and reasonable attorney fees arising out of, directly or indirectly, any claim of personal injury or death associated with such unintended or unauthorized use, even if Elveflow® is allegedly negligent with respect to the design or the manufacture of the product. Care must be taken when the Elveflow[®] pump is pressurized to ensure that the instrument is not damaged in any way.

Protection

Safety glasses and lab coats should be worn at all times when using an Elveflow[®] pressure pump due to the use of pressurized equipment. This is particularly important when hazardous liquids are used.

Electrictity Advice

Use Elveflow® instruments with the provided power unit only. Maintenance should only be attempted by qualified Elveflow® personnel. Removal of the back panel may invalidate any warranty.

Before applying power: verify that the line voltage matches the product's input voltage requirements and that the correct fuse is installed. Use only the specified line cord for this product and make sure the line cord is certified for the country of use.

Fuses: only fuses with the required rated current, voltage, and specified type (normal blow, time delay, etc.) should be used. Do not use repaired fuses or short-circuited fuse holders. To do so could cause a shock or fire hazard.

Keep away from live circuits: operating personnel must not remove instrument covers. Component replacement and internal adjustments must be made by qualified service personnel. Do not replace components with a power cable connected. Under certain conditions, dangerous voltages may exist even with the power cable removed.

To avoid injuries, always disconnect power, discharge circuits and remove external voltage sources before touching components.

ESD precautions: the inherent design of this component causes it to be sensitive to electrostatic discharge (ESD). To prevent ESDinduced damage and/or degradation, take customary and statutory ESD precautions when handling this product.

Maintenance advice

Maintenance should only be attempted by qualified Elveflow® personnel. Removal of the back panel will invalidate any warranty.

Do not service or adjust alone: do not attempt internal service or adjustment unless another person, capable of rendering first aid and resuscitation, is present.

Do not substitute parts or modify the instrument: because of the danger of introducing additional hazards, do not install substitute parts or perform any unauthorized modification to the instrument.

Return the instrument to an Elveflow® Technologies Sales and Service Office for service and repair to ensure that safety features are maintained.

Instruments which appear damaged or defective should be made inoperative and secured against unintended operation until they can be repaired by qualified Elveflow® personnel.

CE compliance

This product meets the essential requirements of applicable European Directives, as amended for CE marking, as follows:

Electromagnetic Compatibility

COUNCIL DIRECTIVE 89/336/EEC of 3 May 1989

This directive has been amended by the following Council Directives:

1. 92/59/eec of 29 June 1992 (General Product Safety)

2. 93/68/eec of 22 July 1993 (CE Marking directive)

3. 99/5/ec: Directive of Radio Equipment & Telecommunications Terminal Equipment (R&TTE).

Technical support

General information <u>contact@elveflow.com</u> +33(0).184.163.807

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www.elveflow.com

<u>Elveflow.com/microfluidic-products/microfluidics-application-packs/easy-droplet-generation/</u>

Product:

MAY THE FLOW BE WITH YOU