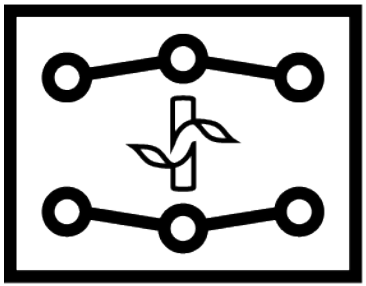
User's Manual



EZ Loop

Recirculation Solution

Contents

Warnings

Do never open the SWITCHBOARD, 2-SWITCH™, FLOW UNIT, MFCS™-EZ, Flowboard and FLOW EZ™ devices. Refer all servicing to after-sales service department (support@fluigent.com).

Prevent any objects or liquids from entering the SWITCHBOARD, MFCS™-EZ, Flowboard and FLOW EZ™, this may cause a short-circuit failure or other malfunction. Failing to respect this advice would:

- Expose you to direct current/voltage in case the device is under voltage which may lead to severe damages
- Void device's warranty
- Discharge our company from any liability regarding physical or device damages

Do not place the product in an unstable location, place the device in a location with a level surface and a strong and stable support.

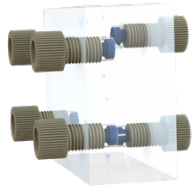
Do not use other power supply than the one provided with the SWITCHBOARD, 2-SWITCH™, FLOW UNIT, MFCS™-EZ, Flowboard and FLOW EZ™ devices. The power supplies have been carefully selected to meet the power requirements in all configurations and to comply with all safety standards.

If you are using the recirculation module with other flow control systems, please check that the pressure in your fluidic system does not exceed 100 psi.

Introduction

The **EZ Loop Recirculation Solution** allows recirculating continuously and for long periods a liquid solution inside a microfluidics chip. Combining the **EZ Loop Module**, two **FLOW EZ™** pressure controllers, **FLOW UNIT** sensor, two **2-SWITCH™** valves, **SWITCHBOARD** device and the **MAT** scripting software will give you the possibility to easily design, build and run simple or complex microfluidic recirculation circuits.

The solution has been designed to work at its best performances with FLUIGENT flow control solutions (FLOW EZ™, MFCS™ and Flow-Rate Platform). It is however possible to use the solution with other flow control systems provided that the pressure applied to the devices **does not exceed 100 psi** (7000 mbar).



The user's manual shows how to use the **EZ Loop** elements for your daily work. It will describe all the **EZ Loop** functionalities that will help you to rationalize your microfluidic circuits and automate your experiments. You will also find answers to the frequently asked questions about the **EZ Loop**. With these elements you will be able to exploit the full performances of the **EZ Loop Recirculation Solution** for your application.

Recirculation characteristics

Benefits & Features

- Control of the flow during several hours, days or weeks
- Accurate and stable flow rates from 0.5 µL/min to 1 mL/min
- Control of the volume & dilution of the recirculated solution

Specifications

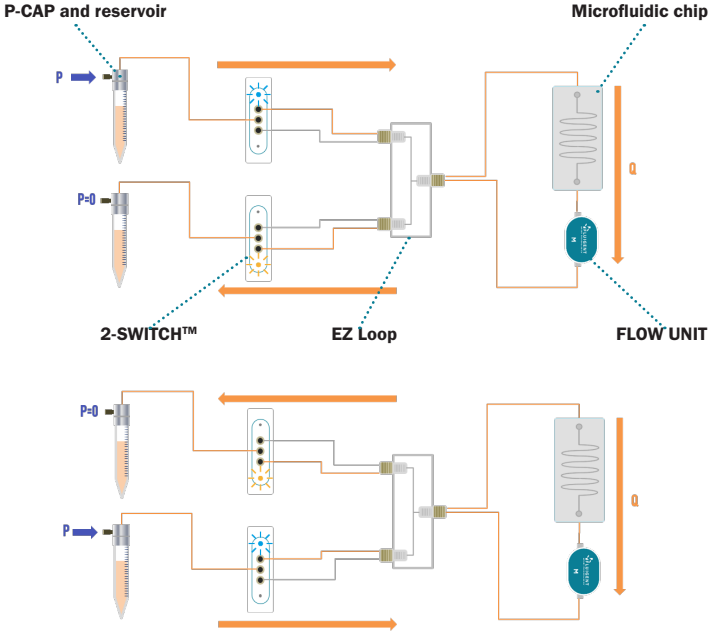
General	
Material	PMMA (transparent)
External dimensions	L * w * h = 50 mm * 40 mm * 20 mm
Weight	47 g
Temperature resistance	Up to 80 °C
Connections	
Connectors	Standard 1/4-28 flat-bottom connectors
Inlets/Outlets	6 fluidic ports - 4 connected to the two 2-SWITCH™ - 2 connected to the microfluidic chip
Performance	
Flow-rate range	From 0.5 µL/min minimum (with FLOW UNIT S) to 1 mL/min maximum (with FLOW UNIT XL)
Flow-rate accuracy	From 100 nL/min (with FLOW UNIT S) to 0.2 mL/min (with FLOW UNIT XL)*
Internal volume	15 µL
Minimum solution volume required for recirculation	200 µL
Chemical resistance for cleaning	
Recommended chemical	Tergazyme®, Ethanol or Isopropanol

**FLOW UNIT accuracy data, depends on the flow rate.*

For more information please check the FLOW UNIT user's manual.

Recirculation circuit

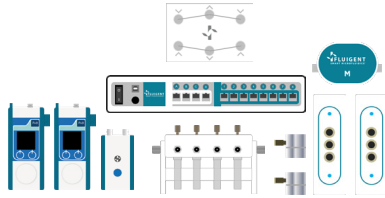
The **EZ Loop** device recirculates your solution alternatively from 2 reservoirs. To do so, the two reservoirs are alternatively pressurized and the two 2-way bidirectional valves controlled in order to redirect the flow inside the microfluidic chip in the same direction.



Contents

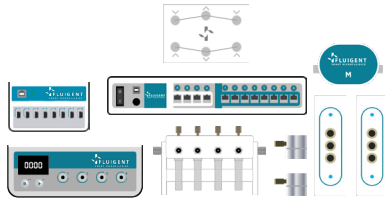
Pack with Flow EZ™ contents:

2 x Flow EZ™
1 x LINK
2 x 2-SWITCH™
1 x SWITCHBOARD
1 x FLOW UNIT M
2 x P-CAPs or Fluiwell
1 x EZ Loop

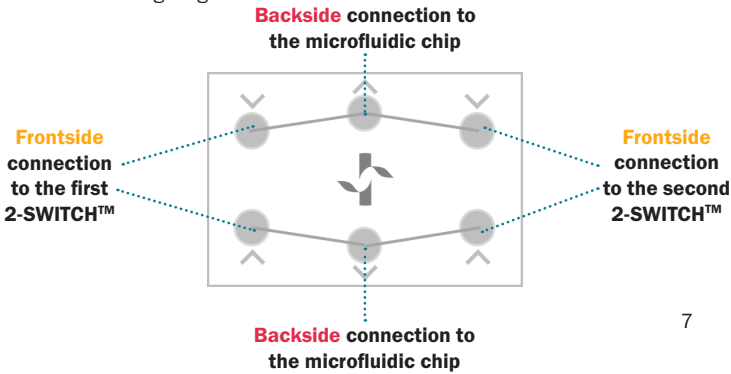


Pack with MFCS™-EZ contents:

1 x MFCS™-EZ
2 x 2-SWITCH™
1 x SWITCHBOARD
1 x FLOW UNIT M
1 x Flowboard
2 x P-CAPs or Fluiwell
1 x EZ Loop



Please note that the pressure range of the pressure controllers, and the FLOW UNIT may change regarding the wanted flow-rate and tubing lengths.



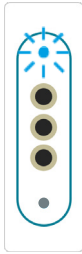
How to start with the EZ Loop

In this section, are explained:

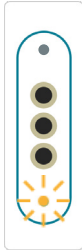
- How to build your microfluidic circuit and connect the instruments
- How to fill the microfluidic circuit with your solution in order to avoid bubbles inside your chip
- How to run your recirculation experiment

NB: In blue quotes, are given indications for a typical recirculation experiment of about 100-500 µL solution with 2 mL Eppendorf reservoirs.

Please note the different 2-SWITCH™ positions as followed



When the 2-SWITCH™ is in position **1**:
The blue LED is lit on

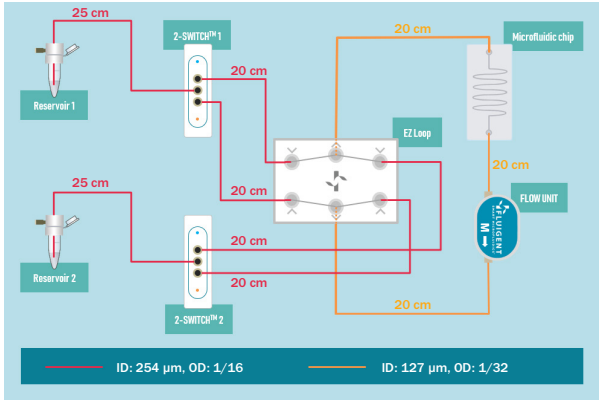


When the 2-SWITCH™ is in position **2**:
The white LED is lit on (represented in yellow)

Build the circuit

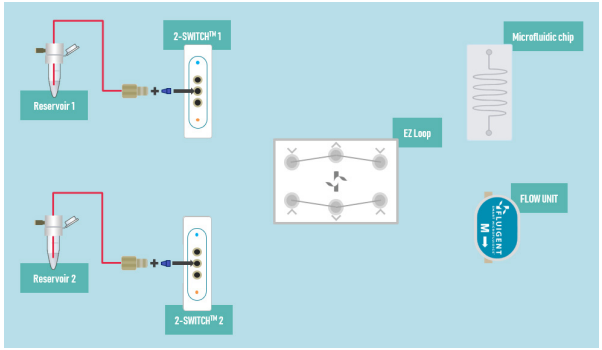
Recirculation fluidic set-up

The following scheme report the tubing dimensions recommended to run an experiment. The lengths are indicative and can be adapted to the set-up. Please note, the shorter the tubing is the lower the internal volume will be.



Fluidic tubing connections

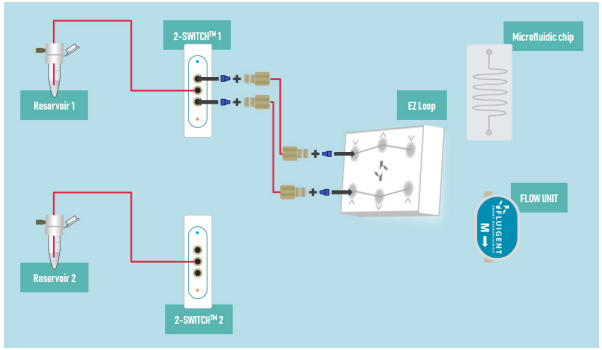
Step 1: Connect the reservoirs to the middle port of the **2-SWITCH™**



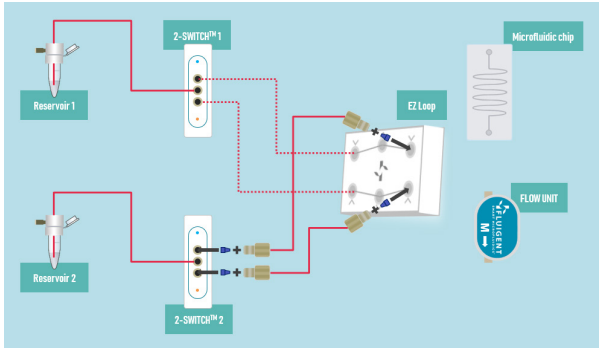
Please refer to the complete recirculation fluidic set-up scheme to know the tubing dimensions (length, inner diameter, outer diameter)

Please pay attention to the flow direction of the **FLOW UNIT***: the arrow on the* **FLOW UNIT** *should indicate the flow direction in the* **microfluidic chip**.

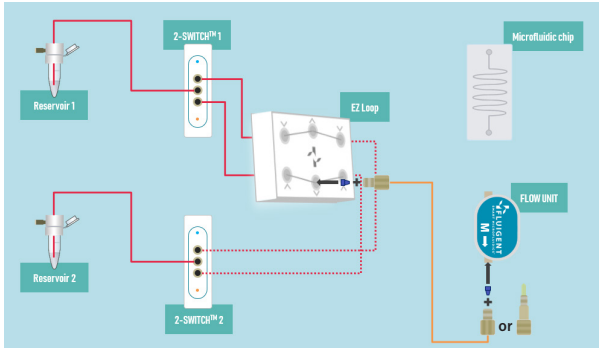
Step 2: Connect the two ports of the **2-SWITCH™** 1 to the **EZ Loop module**



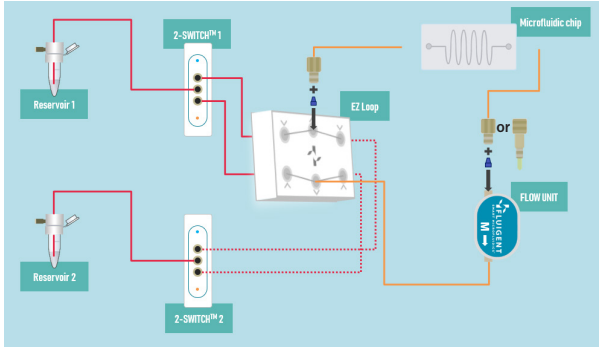
Step 3: Connect the two ports of the **2-SWITCH™** 2 to the **EZ Loop module**



Step 4: Connect the outlet of the **FLOW UNIT** to the **EZ Loop module**



Step 5: Without connecting it to the **microfluidic chip**, connect the tubing to the last outlet of the **EZ Loop module**. Again, without connecting it to the **microfluidic chip**, connect the tubing to the inlet of the **FLOW UNIT.**



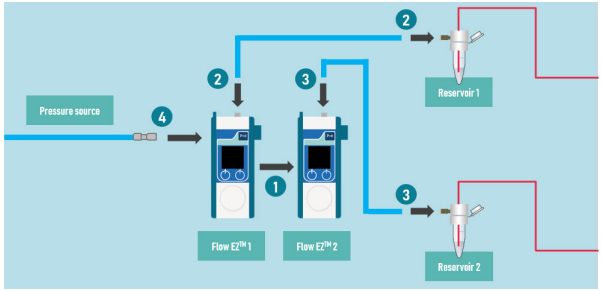
At this stage:

- The two **reservoirs**, the two **2-SWITCH™**, the **FLOW UNIT** and the **EZ Loop** should be connected.
- The **microfluidic chip** should be disconnected, as the fluidic path should be first loaded with liquid before connecting the chip.

Pneumatic connections

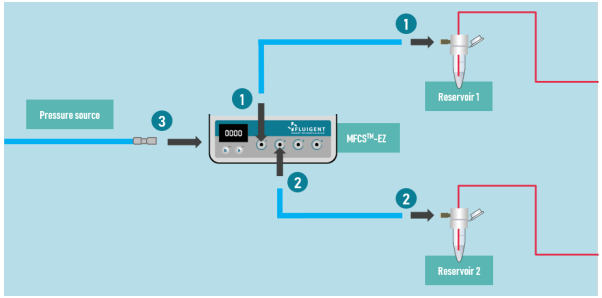
With Flow EZ™ (refer to **Flow EZ™** *user manual* for more information)

1. Connect the two **Flow EZ™** together
2. Connect the **Reservoir 1** to **Flow EZ™** 1
3. Connect the **Reservoir 2** to **Flow EZ™** 2
4. Connection to the pressure source



With MFCS™-EZ (refer to **MFCS™-EZ** *user manual* for more information)

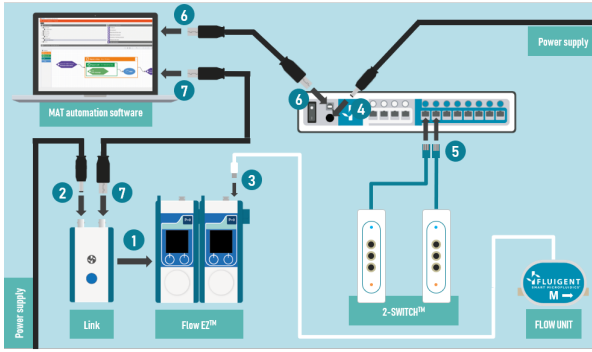
1. Connect the **Reservoir 1** to the channel 1 of **MFCS™-EZ**
2. Connect the **Reservoir 2** to the channel 2 of **MFCS™-EZ**
3. Connection to the pressure source



Electronic connections

With Flow EZ™ (refer to **Flow EZ™** *user manual* for more information)

1. Connect the **Link** to the two **Flow EZ™** for software automation
2. Provide power supply to the **Link**
3. Connect the **FLOW UNIT** to any **Flow EZ™**
4. Provide power supply to the **SWITCHBOARD**
5. Plug the **2-SWITCH™** 1 to the port 1 of the **SWITCHBOARD** and **2-SWITCH™** 2 to the port 2
6. Connect the **SWITCHBOARD** to the computer by USB cable
7. Connect the **Link** to the computer by USB cable

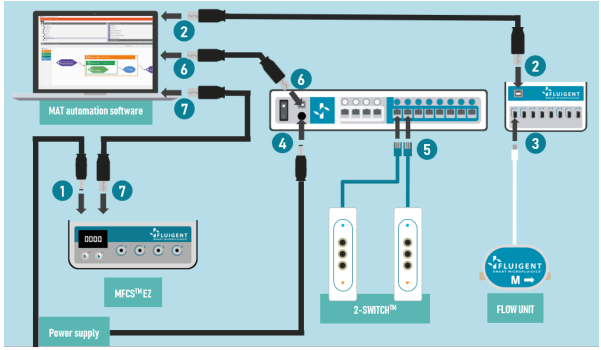


At this stage:

- All the elements are fluidically connected except for the **microfluidic chip**
- The **reservoirs** are pneumatically connected to the **Flow EZ™**
- All the elements are electronically plugged and connected to the PC for automation with **MAT software**

With MFCS™EZ

1. Provide power supply to the **MFCS™-EZ**
2. Connect the **Flowboard** to the computer by USB cable
3. Connect the **FLOW UNIT** to the **Flowboard** on any port
4. Provide power supply to the **SWITCHBOARD**
5. Plug the **2-SWITCH™** 1 to the port 1 of the **SWITCHBOARD** and **2-SWITCH™** 2 to the port 2
6. Connect the **SWITCHBOARD** to the computer by USB cable
7. Connect the **MFCS™-EZ** to the computer by USB cable



At this stage:

- All the elements are fluidically connected except for the **microfluidic chip**
- The **reservoirs** are pneumatically connected to the **MFCS™-EZ**
- All the elements are electronically plugged and connected to the PC for automation with **MAT software**

Load the system with the solution

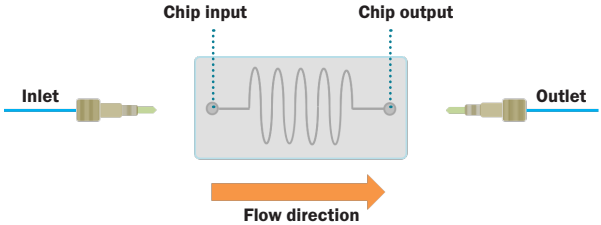
Objective: Flush out the air in the tubing and fill the fluidic circuit with the solution.

Protocol

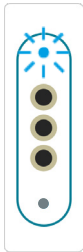
- Fill half of both reservoirs

For 2 mL Eppendorf reservoirs, fill with 1 mL

- Check that the outputs of the microfluidic chip are disconnected as shown on the figure below

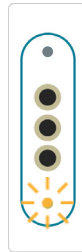


Please note the different **2-SWITCH™** positions as followed



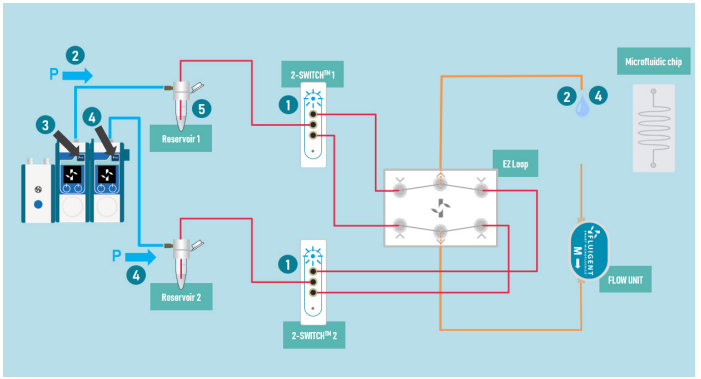
When the **2-SWITCH™** is in position **1**:
The blue LED is lit on

When the **2-SWITCH™** is in position **2**:
The white LED is lit on
(represented in yellow)



Phase 1: Filling inlet paths

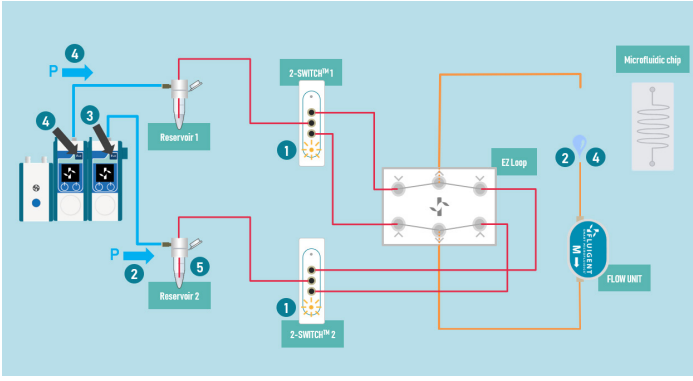
1. Put both **2-SWITCH™** on position **1** (blue LED ON)
2. Increase the pressure of **Flow EZ™** 1 or **MFCS™-EZ** channel 1 until liquid flow out of the inlet
3. Stop the pressure
4. Increase the pressure of **Flow EZ™** 2 or **MFCS™-EZ** channel 2 until all air bubbles are ejected and have a constant flow out of the inlet, and then stop the pressure
5. Check if there still is solution inside **reservoirs** 1 and 2



If a reservoir is empty, you have probably introduced air in the tubing. Fill again the reservoir and restart the phase 1.

Phase 2: Filling outlet paths

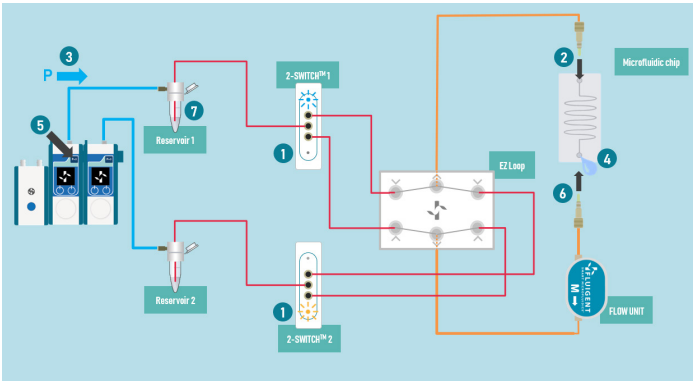
1. Put the both **2-SWITCH™** on position **2** (white LED ON)
2. Increase the pressure of **Flow EZ™** 2 or **MFCS™-EZ** channel 2 until liquid flow out of the inlet
3. Stop the pressure
4. Increase the pressure of **Flow EZ™** 1 or **MFCS™-EZ** channel 1 until all air bubbles are ejected and you have a constant flow out of the inlet, and then stop the pressure
5. Check if there still is solution inside **reservoirs** 1 and 2



If a reservoir is empty, you have probably introduced air in the tubing. Fill again the reservoir and restart the phase 2.

<u>Phase 3:</u> Filling the microfluidic chip with solution

1. Set the **2-SWITCH™** 1 is in position **1** and the **2-SWITCH™** 2 in position **2**
2. Connect the inlet of the **microfluidic chip**
3. Flow your solution inside the **microfluidic chip** by increasing the pressure of **Flow EZ™** 1 or **MFCS™-EZ** channel 1
4. Wait until the solution flows out of the **microfluidic chip**
5. Stop the pressure
6. Connect the outlet of the **microfluidic chip**
7. Check if there still is solution inside **reservoirs** 1 and 2



If a reservoir is empty, some air might have entered the tubing. Disconnect the microfluidic chip and fill again the reservoirs and restart the phase 1.

Set the volumes to recirculate between reservoirs

The recirculation will operate from **reservoir** 1 to **reservoir** 2. It is mandatory to partition the solution between the two reservoirs and to recirculate up to **90% of the total volume** to prevent the formation of air bubbles. The solution can be split as follows:

- Fill up **reservoir** 1 with 50 to 90% of the solution
- Fill up **reservoir** 2 with the remaining 50 to 10% of the solution

For 500 µL of solution, fill up reservoirs 1 and 2 with respectively 450 µL and 50 µL, and recirculate 400µL.

Please note that the residual volume should not be less than 20 µL for a 2-days experiment.

At this stage:

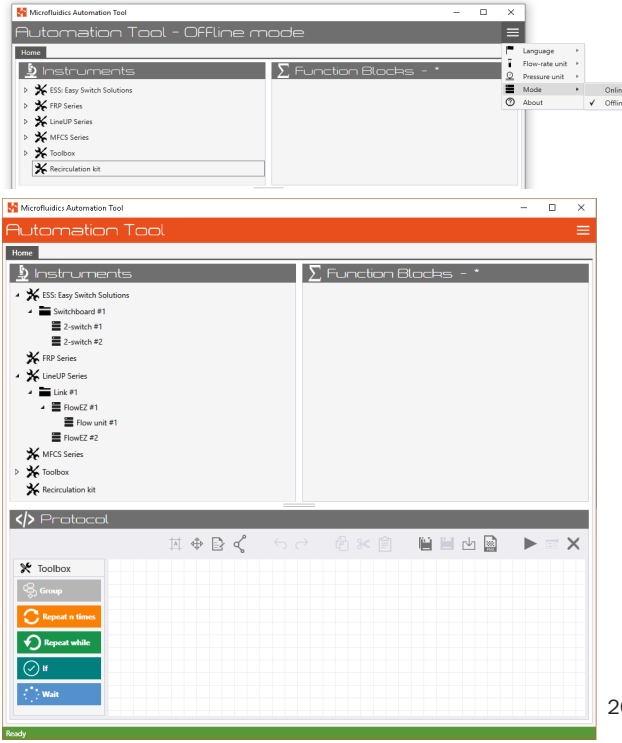
- The **microfluidic chip** is connected to the circuit and all the set-up is filled with liquid and without any bubbles
- The volume in the **reservoirs** is set to start your experiment

Start the experiment with Fluigent MAT software

Objective: Run the recicrculation experiment

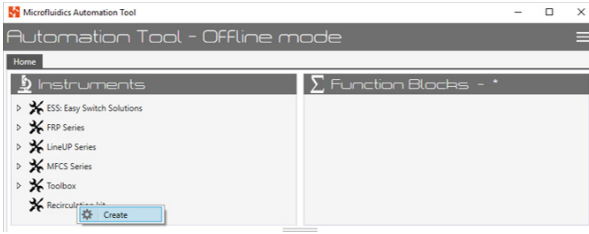
Protocol

- Open the **Fluigent MAT software** (release **19.0.1.1** or higher)
- Select the «Online mode». All the instruments needed, i.e. two **Flow EZ™** or a **MFCS™-EZ**, a **FLOW UNIT**, two **2-SWITCH™**) should appear in the «Instruments» section



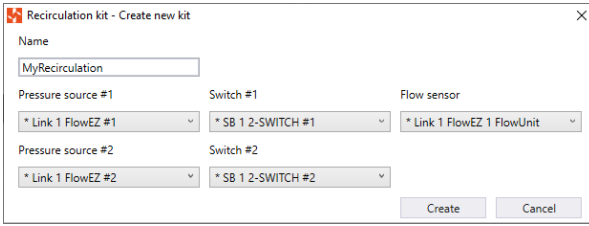
<u>Step 1:</u> Create or edit the recirculation kit

- Click right on **Recirculation kit** in the **Instruments** menu



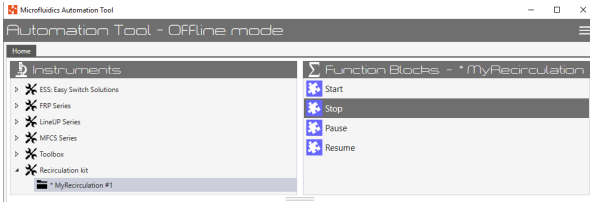
- Click on **Create**
- Fill the information of the recirculation kit according to the layout built

- Give a name to your recirculation kit
- In Pressure source #1, select the pressure channel connected to the **reservoir 1**
- In Pressure source #2, select the pressure channel connected to the **reservoir** 2
- In Switch #1, select the **2-SWITCH™** connected to the reservoir 1
- In Switch #2, select the **2-SWITCH™** connected to the reservoir 2
- In Flow sensor, select the **FLOW UNIT** connected after the **EZ Loop** module



Step 2: Recirculation function blocs

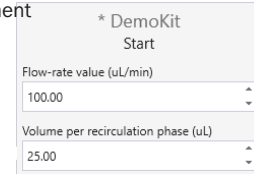
- Once a recirculation kit is created, the recirculation functions appear in the right menu



- Function blocks can be dragged and dropped inside the protocol in order to automate the experiment

Start: Allows one to start the recirculation and specify:

- Flow-rate order
- Volume per recirculation phase



The block triggers the recirculation but does not set the recirculation time. Add a **Wait** block (in the toolbox) to set recirculation time and a **Stop** block to stop the recirculation

Pause: Allows one to pause a previously started recirculation

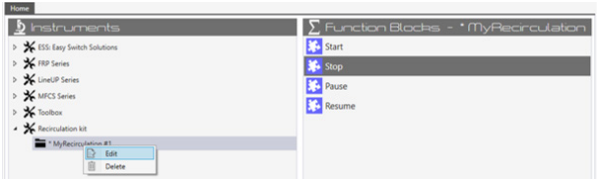
Resume: Allows one to resume a previously started recirculation in the state that it was paused in

Stop: Allows one to stop a previously started recirculation

Step 3: Edit or Delete the recirculation kit

- Right click on «Recirculation kit» in the «Instruments» menu

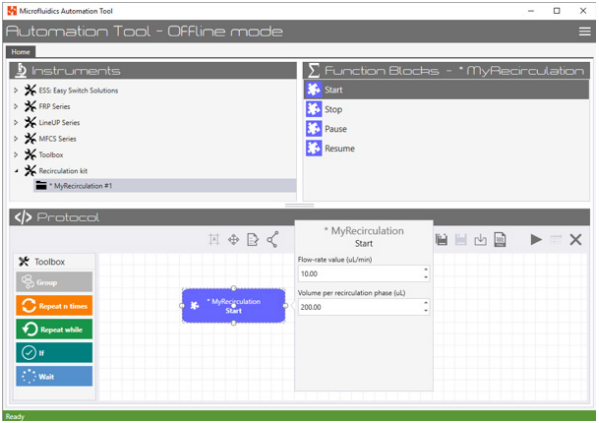
- «Edit» changes the name and instruments of your kit
- «Delete» deletes the recirculation kit



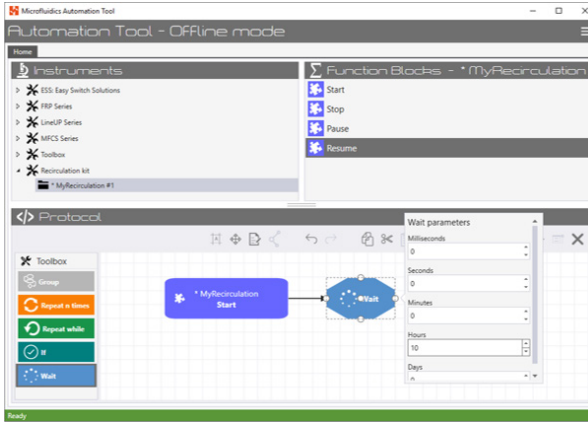
Step 4: Run the recirculation protocol

- Build a protocol for recirculation with **Start**, **Wait** and **Stop** blocks like

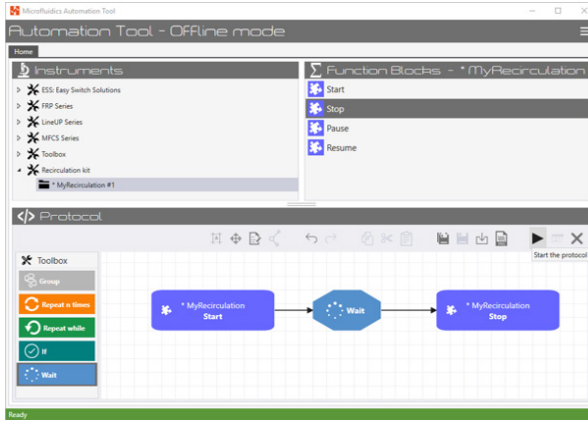
- Drag and drop a **Start** function block and set the flow-rate and the volume to recirculate between the two reservoirs



- Drag and drop a **Wait** function block to set the recirculation time.



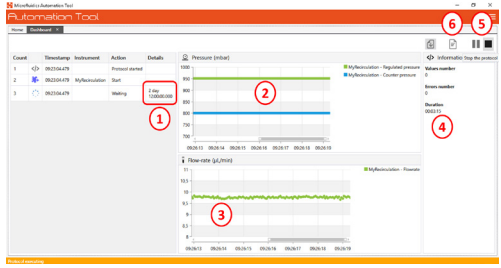
- Drag and drop a **Stop** function block to stop the recirculation



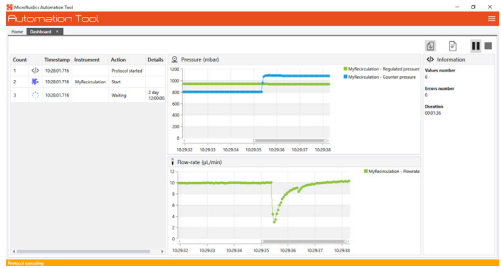
- Press on the **play** button to launch the automated protocol
- The **MAT software** dashboard displays:

- Recirculation protocol on the left with duration information (1) for the wait step
- Pressure (2) and flow rate (3) charts in the middle
- Experiment information and duration on the right (4)
- Control buttons (**Pause** and **Stop**) on the right top corner (5)

- At the end of your protocol, click the file icon on the top right corner (Figure 19) to open the folder where the experimental data are saved (6). The folder is: **C:\Users\user_name\AppData\Local\Fluigent\MAT\Records\Datalog**



During recirculation, when switching recirculation from reservoir 1 to reservoir 2 or vice versa, pressures and flow rate curves shows switch transitions during few milliseconds



Cleaning process

Objective: Clean the instruments and the recirculation module

The protocol consists of flowing:

- Tergazyme® 1% (10 g per litre) at 10 µL/min[1] for 30 min
- DI water 50 µL/min[1] for 10 min
- PA 50 µL/min[1] for 10 min
- Air

Phase 1: Tergazyme® 1%

- Fill half the two reservoirs with Tergazyme® 1% (10 g per L)
- Set a basic **Fluigent MAT software** protocol (with a **Start**, **Wait** and **Stop**)

- Set **Start** *block with 10 µL/min[1] flow-rate and volume superior to the recirculation circuit internal volume*

- Set **Wait** *block to 30 minutes*

Phase 2: Deionized water (DI water)

- Fill half the two reservoirs with DI water
- Change the setting of the **Fluigent MAT software** protocol

- Set **Start** *block with 50 µL/min[1] flowrate and volume superior to the recirculation circuit internal volume*
- Set **Wait** *block to 10 minutes*

Phase 3: Isopropanol (IPA)

- Fill half the two reservoirs with IPA
- Change the setting of the **Fluigent MAT software** protocol

- Set **Start** *block with 50 µL/min[1] flowrate and volume superior to the recirculation circuit internal volume*
- Set **Wait** *block to 10 minutes*

Phase 4: Air

- Empty the two reservoirs
- Put the **2-SWITCH™** 1 and **2-SWITCH™** 2 on position 1 (blue LED ON)
- Increase the pressure of **Flow EZ™** 1 or **MFCS™-EZ** channel 1

- Liquid flow out inside the reservoir 2
- Wait until air flows out

- Put the **2-SWITCH™** 2 on position 2 (white LED ON)

- Liquid flow out inside the reservoir 2
- Wait until air flow out

- Put the **2-SWITCH™** 1 on position 2 (white LED ON)

- Liquid flow out inside the reservoir 2
- Wait until air flow out

- Put the **2-SWITCH™** 2 on position 1 (blue LED ON)

- Liquid flow out inside the reservoir 2
- Wait until air flow out

- You can disconnect your microfluidic circuit and tidy up the experimental setup

[1] These values are ideal conditions but the flow-rate may depend on your **FLOW UNIT**. For example with **FLOW UNIT S**, set the value to 5 µL/min instead of 50 or 10 µL/min.

Frequently Asked Questions

How much should I fill the reservoirs?

First of all, both reservoirs should be filled with your solution. Indeed, despite there is recirculation from reservoir 1 to the reservoir 2 and the same amount of liquid will recirculate back to the reservoir 1. It is mandatory to fill a minimum amount of solution in the reservoir 2 in order to avoid insertion of air bubbles in the circuit. The minimum is 20 µL with flow rate up to 10 µL/min, but this volume can be higher if you use a flow rate superior to 0.1 mL/min.

Case 1: Fill the two reservoirs with the same volume

- Fill up the two reservoirs with the same volume of your solution

- Maximum value: half capacity of the reservoir (1 mL for 2 mL Eppendorf reservoirs)
- Minimum value: 200 µL

Example case: You have 2 mL Eppendorf reservoir and 500 µL of solution. Fill up both reservoirs 1 and 2 with 250 µL.

Case 2: Avoid dilution of the cell secretions

- Fill up reservoir 1 with 90% of your solution

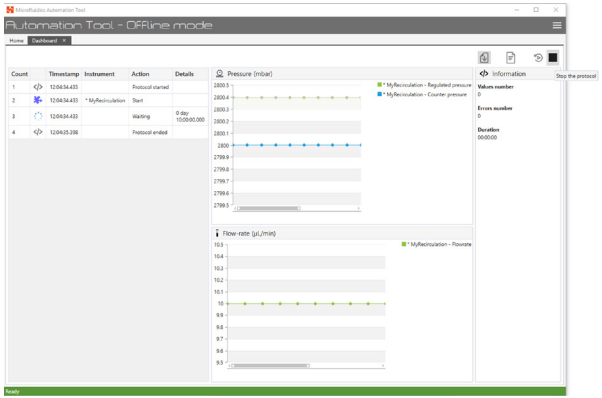
- Fill up reservoir 2 with the remaining 10% of your solution
- Minimum value: 20 µL for experiment of 2 days maximum since some evaporation could occur

Example case : You have 500 µL of solution. Fill up reservoirs 1 and 2 with respectively 450 ul and 50 µL.

Check the part «**Set the volumes to recirculate between reservoir**»

Can I stop the MAT protocol and change the solution as I wish ?

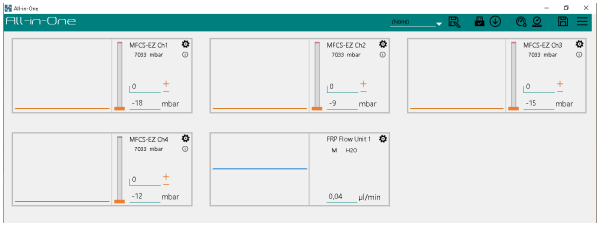
After running the recirculation experiment thanks to **Fluigent MAT software**, stopping the experiment is possible whenever wished despite the **MAT** protocol is not finished. To stop the experiment click on the **Stop** button on the upper right corner on the «Dashboard» tab of the **MAT** software.



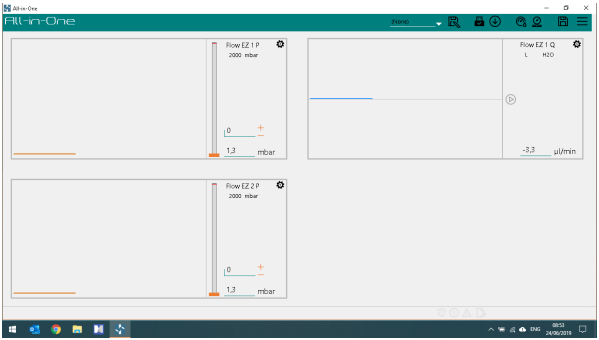
It is possible to restart the recirculation protocol from the protocol window or the button **Restart** near the **Stop** button. However, previously, check the volumes of solution inside the two reservoirs and adjust them manually as they were at the beginning.

When manually filling the recirculation circuit, how do I increase the pressure of MFCS™-EZ pressure controller ?

With **MFCS™-EZ** pressure controller solution, use **Fluigent A-i-O software** solution. Connect the **MFCS™-EZ** and the **Flowboard** to the PC and open **Fluigent A-i-O**. One can control the pressure of the channels through the orange sliders.



With **LineUP Flow EZ™**, one can either control the pressure manually, either with **Fluigent A-i-O.** For live control with the PC, connect the two **Flow EZ™** to the PC, open **Fluigent A-i-O** and control the pressure of the channels through the orange sliders.



Warranty terms

What This Warranty Covers

This warranty is granted by Fluigent and applies in all countries.
Your Fluigent product is guaranteed for one year from the date of delivery at your laboratory against defects in materials and workmanship.
If found to be defective within the warranty period, your Fluigent product will be repaired or replaced free of charge.

What This Warranty Does Not Cover

This warranty does not cover routine maintenance, or damage resulting from the failure to maintain the product in accordance with instructions provided by Fluigent. This warranty also does not cover damage that arises from accidental or intentional misuse or abuse, alteration or customization, or repaired by unauthorized persons.

How to Get Service

If something goes wrong, contact the Fluigent dealer from whom you purchased your product. Arrange a mutually convenient time for Fluigent service representative to discuss over the problem and find a solution to fix the issue. Will be favored any remote repairs, but in case more actions need to be taken, the system will come back to Fluigent offices (for no additional cost, only if it is under warranty).

The warranty conditions are:

- Do never open the SWITCHBOARD, 2-SWITCH™, FLOW UNIT, MFCS™-EZ, Flowboard and FLOW EZ™ devices.
- Do not use other cables than cables provided by Fluigent.
- Prevent foreign objects or liquids from entering the SWITCHBOARD, MFCS™-EZ, Flowboard and FLOW EZ™
- Prevent foreign objects from entering the 2-SWITCH™, FLOW UNIT, MFCS™-EZ, and FLOW EZ™ devices.
- Connect the power cord to an AC outlet of the correct voltage.
- Use the right RJ45 port on the SWITCHBOARD with the right device.
- Do not place the product in an unstable location, place the unit in a location with a level surface and a strong and stable support.
- If you are using the ESS™ platform with other flow control systems, please check that the pressure in your fluidic system does not exceed 100 psi (or 7000 mbar).
- Respect the temperature compatibility (from 5 °C to 50 °C).
- Use the specific connectors provided by Fluigent (Teflon fittings and nuts for the 2-SWITCH™).
- Only use 1/16'' or 1/32" (with 1/16" sleeves) OD tubing with the valves (each type).

For specific use, please contact our Support team at support@fluigent.com

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Technical support

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