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Campus Río Ebro  
Mariano Esquillor s/n Edificio CEMINEM  
50018, Zaragoza, Spain

info@beonchip.com  
+34 656 23 25 63  
+34 655 16 16 91  
www.beonchip.com

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BE-FLOW is a versatile microfluidic device for cell culture under biomimetic conditions. It does in vitro simulation of physiological environments involving flow and shear stress. Long-term 2D or 3D experiments in three independent channels under flow conditions.

Examples of applications are processes related to cell adhesion to the vascular endothelium (infection, cell therapy, metastasis, etc.).

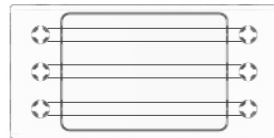
For further information, please visit <https://beonchip.com/be-flow/> or contact BEONCHIP

### MATERIAL

BE-FLOW chips are made of biocompatible plastic and are gas-impermeable, for effective gradients of CO<sub>2</sub>, O<sub>2</sub>, etc. They have excellent optical properties, with high transparency and low auto-fluorescence.

### TECHNICAL FEATURES

	Channel
Number of channels	3
Height	200 µm
Width	1200 µm
Total volume	6 µL
Length	24,6 mm
Total area	29,5 mm <sup>2</sup>



### CONTENT

The product reaches the user sterilized (10 Be-Flow per box). It can be stored in dry places which are not exposed to direct sunlight at room temperature (15-25°C).

### CELL CULTURE COATING

BE-FLOW chips have been treated to obtain a hydrophilic surface that facilitates filling the devices with aqueous solutions and/or gels and promotes cell adhesion.

In case of a certain coating is required, prepare your coating solution (Collagen I, Collagen IV, Fibronectin, Poly-L-Lysine, Poly-D-Lysine...) according to the manufacturer's instructions and apply it into each channel. Aspirate the channel and wash with distilled water to remove excess coating solution by using 5-10 times the volume of the channel.

## FILLING AND HANDLING

1. Place the chip in a sterile petri dish with lid for handling.
2. Fill channels with a pipette of 100, take 50 ul of culture medium with cells and insert the pipette into the inlet.
3. Carefully fill the channel until you reach the outlet. It is important to leave culture medium both in the area of the entrance and the exit to avoid evaporation. In addition, it is advisable to add distilled water in the upper hole of the chip to control evaporation in the device.
4. Let cells adhere to the surface (the time may vary depending on the cell type).
5. If you are going to leave the device without mounting the flow system on the same day of cells seeding, it is advisable to change the medium every 24 hours. To do this, medium is added carefully in the inlet and the excess is collected by the exit. In this way the new medium replaces the old one.

## ASSEMBLY OF THE FLOW SYSTEM

Beonchip has a microfluidic starter kit to set different flow system configurations.

Previous considerations:

1. Set the system in a laminar flow cabinet.
2. Cells must be well adhered to the surface before mounting the flow system.
3. The device should be never left without culture medium inside or in the inlets / outlets.

To assemble the flow system the following will be considered:

1. Fill the inlet area completely with medium so that no air bubbles remain.
2. Prime the system of tubes that reach the inlet before assembling the system.
3. Both inlets and outlets are designed to be able to connect a tube with an outside diameter of 2.4 mm without the need to use connectors. Once this tube system is primed, the tube can be inserted into the inlet. At this point, extreme care must be taken to ensure that no air bubbles enter the system.
4. Finally, the tube is connected to the outlet and thus the system is closed.
5. Check that there are no leaks in the system. To do this, leave the pump running for a couple of minutes before placing the devices in the bioreactor or in the incubator.

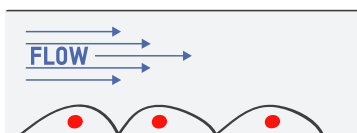
To view a video about filling a BE-FLOW device, please visit <https://beonchip.com/be-flow/>



To prevent bubbles from forming during filling, please avoid empty completely tips of pipettes. Hold the plunger firmly while removing the pipette from the inlets so that the negative pressure will not suck the solutions up.

## CELLS SEEDING

Interaction between hemodynamics and vascular endothelium is an important factor to consider in cardiovascular function. Vascular endothelial cells from the inner wall of arterial vessels are exposed to the blood flow which acts on the endothelium surface as a tangential force (shear stress). Under mechanical stimuli, cells can adjust several biological responses through mechanotransduction processes. The BE-FLOW design consists of three independent channels. Each channel has an inlet and outlet reservoir that can be connected with a flow system, emulating the frame of a blood vessel. Seeding endothelial cells on top of a ECM coated surface, that meet cell type specific needs for growth and adhesion, makes possible to recreate a dynamic environment under different, physiological and pathological, flow rates.



## PREPARATION FOR CELL MICROSCOPY

It is possible to monitor fixed or living cells and also chemical gradients. Most of the monitoring systems used in traditional cell culture can be taken to BEONCHIP microfluidic devices. Common fixatives can be used. Cell viability can be evaluated using different dyes. Moreover, immunofluorescent staining can be performed to identify specific targets. Also, cell cycle fluorescent reporters can be used.

*Please contact BEONCHIP for further assistance.*

## OTHER READOUTS

It is possible to recover cells and perform flow cytometry, RNA extraction (PCR), exosomes...

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