

BE-FLOW is a slide format 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 two independent channels under flow conditions.

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

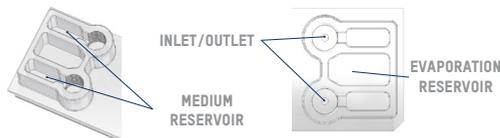
For further information, please contact BEONCHIP

MATERIAL

BE-FLOW chips are made of biocompatible plastic and are gas-impermeable, for effective gradients of CO₂, O₂, etc. They have excellent optical properties, with high transparency and low auto-fluorescence.

TECHNICAL FEATURES

The design of the be-flow consists of 2 independent channels with screw-like inlet and outlet wells that allow joining with connectors and tubes to a fluidic system. Evaporation reservoirs are next to the medium reservoirs to be filled with PBS or water during the incubation before closing the system with tubes.



	Height	Width	Length	Total Volume
Channels	375 µm	1'5 mm	43 mm	24 µL
Inlet/Outlet	7 mm	UNF 1/4" - 28		130 µL
M. Reservoir	5 mm	3'6 mm	8'8 mm	185 µL



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 or PBS to remove excess coating solution by using 5-10 times the volume of the channel.

FILLING AND HANDLING

1. Trypsinize and count cells as usual. Cell concentration will vary with the cell type. It is recommended to seed a cell concentration to obtain a confluent layer within 2-3 days.
2. With a P-100 or P-1000 pipette, take 100 μ L of medium with cells and add it in the inlet. Leave the medium flow through the channel letting it reach the outlet. It is important to add water or PBS in the evaporation reservoirs to avoid evaporation.
3. Let cells adhere to the surface (the time may vary depending on the cell type).
4. If you are not mounting the flow system on the same day of seeding, add the desired amount of medium in the medium reservoirs. Depending on your cell type, pay attention to change the medium. To do this, medium is added carefully in the medium reservoir next to the inlet and the pressure difference will equalise the medium among inlet and outlet.
5. In order to connect the device to a flow system, screw the connectors into the outlet and inlet (in that order). Both inlets and outlets are designed to be screwed to connectors (1/4" - 28). Remember that the device and the tubes have to be primed with medium before connecting.

It is possible to use a rocker to move medium from one reservoir to another. With a final volume of 100 μ l per reservoir it is possible to apply 45° of inclination.

ASSEMBLY OF THE FLOW SYSTEM

Beonchip has some advices to set the flow system configuration.

Previous considerations:

1. Prewarm the tubes and the fluidic elements during 15-20 minutes at the incubator.
2. Set the system in a laminar flow cabinet.
3. Cells must be well adhered to the surface before mounting the flow system.
4. 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 well completely with medium so that no air bubbles remain.
2. Prime the system of tubes that connect to the inlet before assembling the system.
3. Both inlets and outlets are designed to be able to use connectors (1/4" - 28). Once this tube system is primed, the connector can be screwed 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 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

Recovering the cells is possible in order to perform downstream applications such as flow cytometry, RNA extraction (PCR)...

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