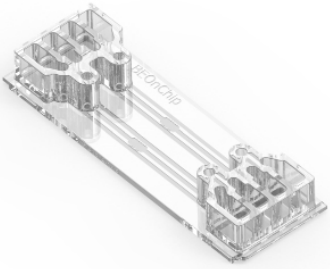
BE-GRADIENT
TECHNICAL CHARACTERISTICS



BE-GRADIENT is a versatile microfluidic device for cell culture under biomimetic conditions. It allows to perform cell cultures under chemical gradients. The optical transparency of the polymers used make possible monitor experiments with phase contrast, fluorescence and confocal microscopy.

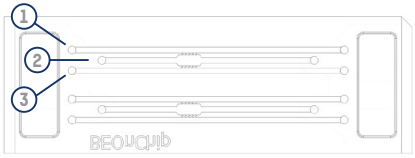
Examples of applications are cell/spheroid invasion and migration, angiogenesis, metastasis, vasculogenesis, chemotaxis, ischemia, cell differentiation or oxidative stress.

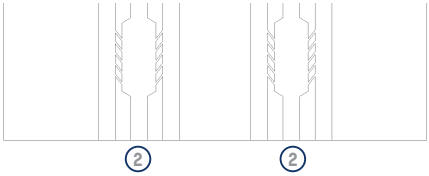
For further information, please visit https://beonchip.com or contact BEONCHIP

MATERIAL

BE-GRADIENT chips are made of biocompatible plastic and are gas-impermeable, for effective gradients of CO_2, O_2, etc. They have excellent optical properties, with high transparency and low auto-fluorescence.

TECHNICAL FEATURES



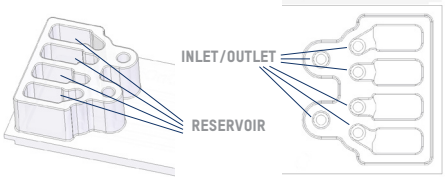


Channel	Height	Width	Lenght	Total Volume
Lateral 1	300 µm	1 mm	50 mm	14,5 µL
Central 2	300 µm	1 mm	39 mm	12,6 µL
Lateral 3	300 µm	1 mm	50 mm	14,5 µL

	Height	Length	Width	Volume
Chamber 2	300 µm	4'6 mm	2 mm	3 µL
Inlet/Outlet	8 mm	Ø = 2'3 mm		18'4 µL
Reservoir	6 mm	3'6 mm	7 mm	151'2 µL

CONTENT

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



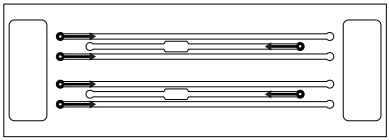
CELL CULTURE COATING

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

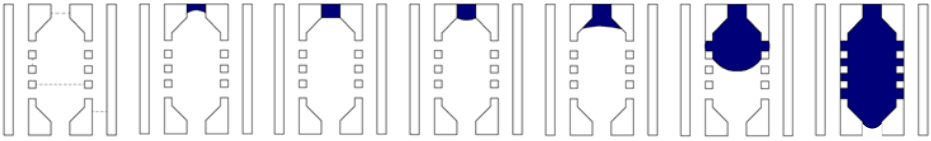
In case of a certain coating is required, prepare your coating solution (Collagen I, Collagen IV, Fibronectin, Poly-L-Lysine, Poly-D-Lysine…) according to the manufacturer's instructions and apply it into the central channel. Aspirate the channel and wash with distilled water to remove excess coating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

FILLING AND HANDLING

1. Fill the channels form the inlets specified, making sure the direction of the flow corresponds with the direction indicated by the arrows (A).



A



B C D E F G H

2. In order to confine the chip it must be completely dry inside the chamber (B).

3. With a P-100 pipette, take from 30 to 50µL of solution to be confined. It is advisable to use an hydrogel with cells in the central channel and insert the tip of the micropipette in the central inlet.

4. Slowly press the plunger to inject the solution into the chip (C, D). First, the meniscus will enter into the chamber (E, F). Continue until the central channel is completely filled (G, H).

5. Place a drop of medium into the central channel inlet and outlet to avoid evaporation during handling.

If it is a 3D cell culture, at this point it is recommended to reverse the chip every 10-15 seconds for 2-3 minutes in order to obtain an homogeneous cell distribution and prevent the cells from settling on the bottom of the chip. Then, place the chip in the incubator for 30 min at 37ºC or until the gel polymerization is completed.

In case that it is a 2D cell culture, leave the cells attach to the surface (under conditions required) before filling the lateral channels. The time required will vary with the type of cells.

Once the central channel is filled, proceed with the lateral ones as follows:

6. With a P-100 or P-1000 pipette, take 200 µL of medium and add it in the reservoirs placed at the same side. Leave the medium flow through the channel letting it reach the opposite reservoir. Pay attention to fill a reservoir and leave the reservoir at the end of the same channel empty. The flow will be equal in both reservoirs, having the same medium volume. It is possible to use a rocker to move medium from one reservoir to another. With a final volume of 100 µl per reservoir it is possible to apply 45° of inclination.

7. In order to connect the device to a flow system, link the tubes into the channel inlets and outlets. Both inlets and outlets are designed to be connected to a tube with an outside diameter of 2.4 mm. Remember that the device and the tubes have to be primed with medium before applying flow. After the tubes are linked, the system is completely isolated and you can remove medium from reservoirs.

To prevent bubbles from forming during filling, please firstly prewarm the device and 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.

ASSEMBLY OF THE FLOW SYSTEM

Beonchip has some advices to set the flow system configuration.

Previous considerations:

1. Prewarm the tubes and the external reservoir 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 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.

CELLS SEEDING

For culturing in 3D environment, an hydrogel mixture containing cells needs to be prepared.

The following references detail the protocols for seeding BE-GRADIENT devices:

1. Ayuso JM, Monge R, Martínez-González A, Virumbrales-Muñoz M. et al. (2017) Glioblastoma on a microfluidic chip: Generating pseudopalisades and enhancing aggressiveness through blood vessel obstruction events. Neuro Oncol 19 (4): 503-513. doi: 10.1093/neuonc/now230

2. Ayuso JM, Virumbrales-Muñoz M, Lacueva A, Lanuza PM, Checa-Chavarria E, Botella P. et al. (2016) Development and characterization of a microfluidic model of the tumour microenvironment. Sci. Rep. 6, 36086; doi: 10.1038/srep36086 (2016)

3. Ayuso JM, Basheer HA, Monge R, Sánchez-Álvarez P, Doblaré M, Shnyder SD, et al. (2015) Study of the Chemotactic Response of Multicellular Spheroids in a Microfluidic Device. PLoS ONE 10(10): e0139515. doi:10.1371/journal.pone.0139515

It is possible to use BE-GRADIENT device with cells on 2D surfaces under chemical gradients. Please contact BEONCHIP for further assistance.

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…

Please contact BEONCHIP for further assistance.

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Biomimetic Environment On Chip

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