BEOnChip

Biomimetic Environment On Chip

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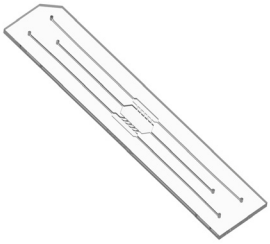
Version 1.0

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.

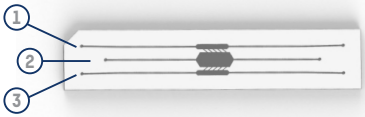
Examples of applications are cell/spheroid invasion and migration, angiogenesis, metastasis, vasculogenesis, chemotaxis, ischemia, cell differentiation or oxidative stress.

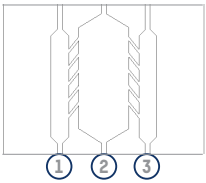
For further information, please visit https://beonchip.com/be-gradient/ or contact BEONCHIP

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





	Height	Total Volume
Lateral 1	300 µm	3 µL
Central 2	300 µm	4 µL
Lateral 3	300 µm	3 µL

Chamber	Height	Volume
Lateral 1	300 µm	1 µL
Central 2	300 µm	3 µL
Lateral 3	300 µm	1 µL

CONTENT

The product reaches the user sterilized and encapsulated in a Petri dish. There are three BE-GRADIENT devices per Petri dish. It can be stored in dry places which are not exposed to direct sunlight at room temperature (15-25ºC).

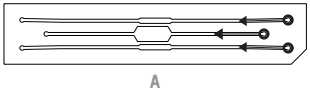
CELL CULTURE COATING

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

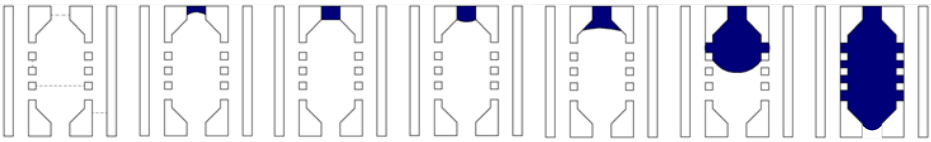
In case of a certain coating is required, prepare your coating solution (Collagen I, Collagen IV, Fibronectin, Poly-L-Lysine, Poly-D-Lysine…) according to the manufacturer's instructions and apply it into the central channel. Aspirate the channel and wash with distilled water to remove excess coating 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 20 µL of solution to be confined, it is advisable to use a gel in the central channel, and insert the tip of the micropipette in the central inlet.

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

5. Place a 5-10 µL drop onto the central channel inlet to avoid evaporation during handling.

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

If the experiment is a 2D cell culture, leave cells to attach 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 pipette, take 40 µL of solution and insert the tip in the lateral channel inlet.

7. Carefully press the plunger until the channel is completely filled and the solution slightly overflows at the outlet.

8. Place a 5-10 µL drop onto lateral channel inlets and outlets, to avoid evaporation during handling.

It is recommended to refresh the medium every 24 hours after cell seeding (or earlier, depending on the experiment). For that, aspirate the drops in inlets and outlets of lateral channels with a P-100 pipette. Then the content of the channel is displaced by carefully adding new solution as described in steps 6-7. Finally, place drops onto inlets and outlets, as described in step 8.

To view a video about filling a BE-GRADIENT device, please visit https://beonchip.com/be-gradient/

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

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

The following references are provided, in which detailed protocols on seeding BE-GRADIENT devices are described (also available for download on the web www.beonchip.com):

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.