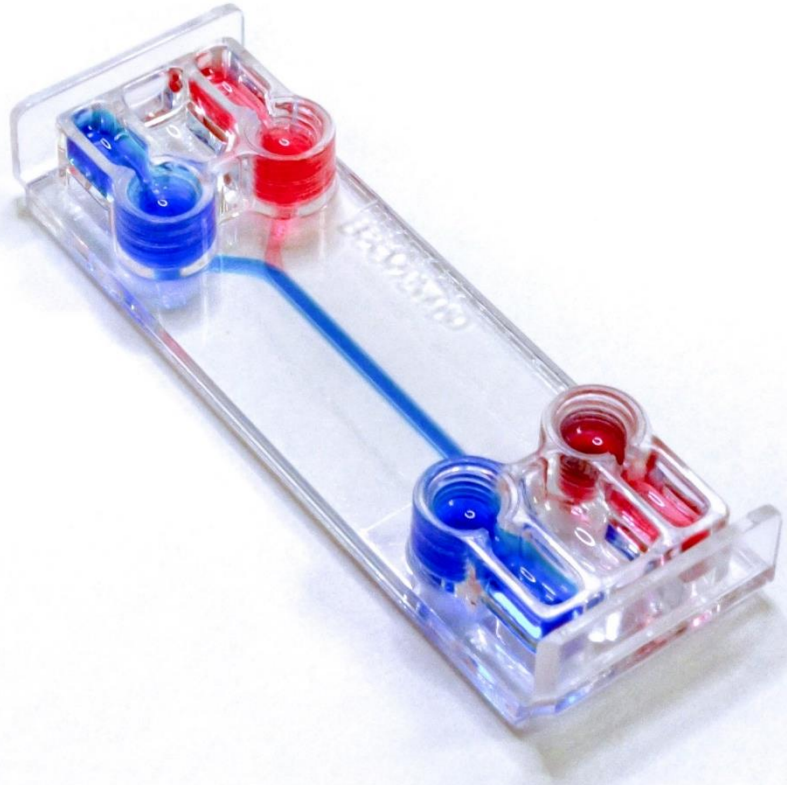
BEOnChip
Biomimetic Environment On Chip

BE-DOUBLEFLOW



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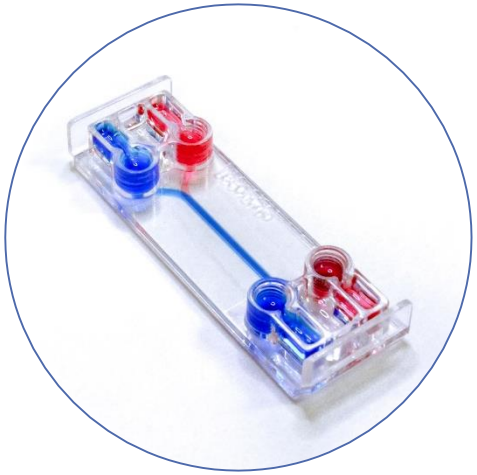
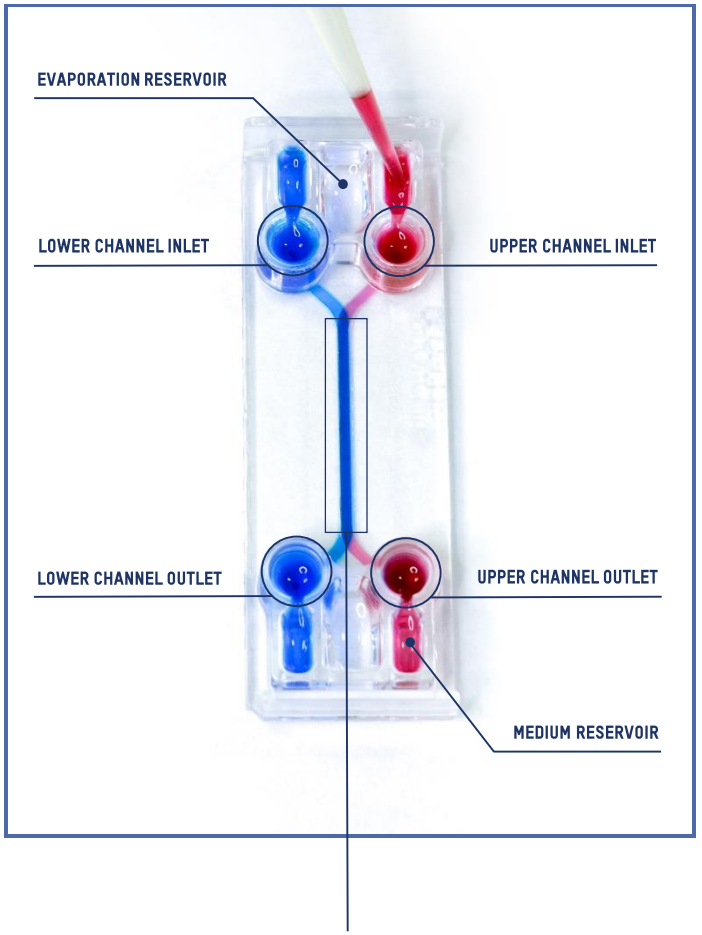


This is our **most advanced** device. It consists of **two perfusable channels connected via a porous membrane**. Explore the crosstalk between different **2D and 3D cultures in a biomimetic environment** and **control** the efficiency of the interaction by selecting the **optimal pore size** for your application. This is the optimal device when a **hypoxia environment** is needed, for studying **the effect of circulating particles** (bacteria, immune system, circulating tumor cells) and for **endothelium/epithelium barrier** when flux plays a role in both sides of the coculture.



TECHNICHAL FEATURES

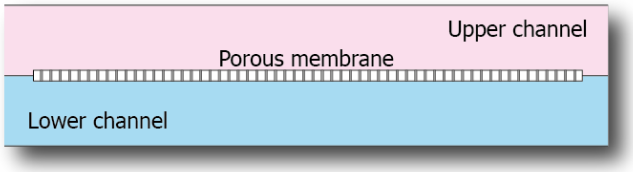
The design of the BE-Doubleflow consists of **2 channels connected through a porous membrane**. Flow is possible in both channels with the help of screw-like designed inlet and outlet wells by screwing them with connectors and tubes to a fluidic system. Evaporation reservoirs are next to the medium reservoirs to be filled with PBS or water during the incubation before closing the system with tubes.

CONTENT

The box contains **10 BE-Doubleflow** chips that are **individually packaged.** Each chip undergoes a sterilization process before sending the chip to the final user. Chips should be stored at room temperature in **dry places** which are **not exposed to direct sunlight** at room temperature **(15-25ºC)**.

	Height	Width	Lenght	Total volume
Each channel	375 µm	1,5 mm	46 mm	31,2 µL
Inlet/Outlet	7 mm	UNF 1/4" - 28		130 µL
Medium reservoir	5 mm	3,6 mm	8,8 mm	185 µL
Membrane pore size	1 µm			

** The volumes presented on the table are theoretical values calculated for the standard products. Changes in the device features of custom chips may modify the exact channel volume.*



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FILLING AND HANDLING

Before seeding, **prewarm the device** in the incubator overnight to avoid the appearance of air bubbles.

When filling the channels up, **place the tip of the pipette completely vertical** into the channel's inlet pinhole. Inject the liquid with a **continuous and constant flow** to avoid backward flow.

Use the **lateral holders** for easy and safe handling.

COATING

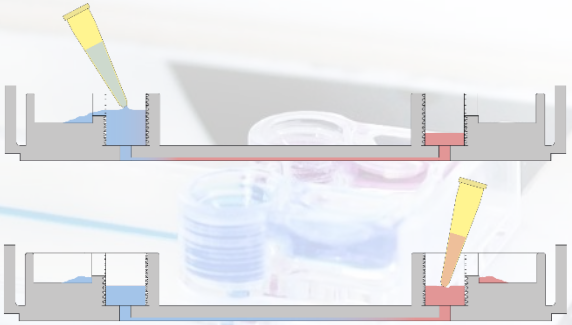
The following protocol is applicable for channel **coating, washing, staining, cell extraction and medium refreshing.**

1. Fill the channel volume up with the **coating solution** by pipetting **through the pinhole** and **incubate** at manufacturer's conditions.

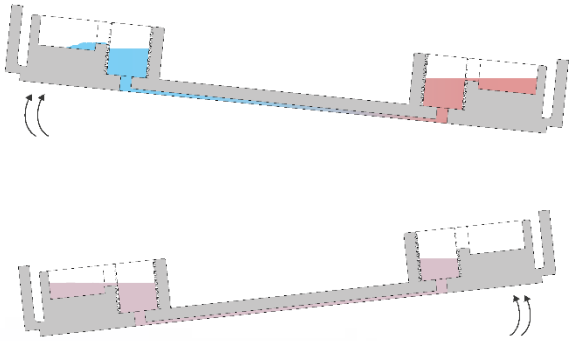
Note: Another way to proceed is to **pipette the solution into the inlet well** and **let the liquid to flow** by itself through the channel **until it reaches the outlet well.**

Tip: When seeding a monolayer underneath the membrane, flip the chip **downwards** and incubate for better coating deposition on the membrane. In this case the volume must be the one that fits in the channel.

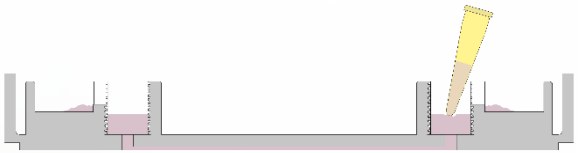
2. Remove the solution from the reservoirs and inlet/outlet wells and **wash the channel** by adding the recommended dilution buffer into the inlet well and removing it at the outlet channel with the pipette. **Repeat** this step as many times as necessary.



Tip: You may **tilt the device** manually in order to make the dilution buffer flow **several times** from one reservoir to another before you remove it.



3. Aspirate the dilution buffer completely before seeding.



Important: Note that the channel should never dry up. Take incubation times into account and use the evaporation reservoirs as long as possible.

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CELL SEEDING/CELL CULTURE

1. Trypsinize and count cells as usual. Cell concentration will vary with cell type and channel dimensions. It is recommended to seed a cell concentration to **obtain a confluent layer within 2-3 days.**

Seeding underneath the membrane

2. Fill the lower channel up with the exact volume of the channel by pipetting through the pinhole. Find the **exact volume** of your chip in the table of the technical feature section.

Important: Adding the **correct volume** into the channel during seeding **helps attaching cells** to the lower side of the membrane.

3. Cover the inlets, **flip the chip downwards** and incubate until the cells attach (time may vary depending on the cell type).

4. After cell attachment, **add more medium** to the medium reservoirs. **Cover and incubate** until needed.

3D culture: In case of **seeding a hydrogel, flip the chip** upwards and downwards **every 10 seconds** for a few minutes (depending on the hydrogel polymerization time) for **homogeneous 3D distribution**.

3D culture is possible to perform both in upper and lower channel. BEOnChip recommends **seeding hydrogels into the lower channel** for better visualization of cells under inverted **microscopy**.

Seeding on the membrane

5. Fill the upper channel up with the exact volume of the channel by pipetting through the pinhole. Find the **exact volume** of your chip in the table of the technical feature section.

Important: Adding the **correct volume** into the channel during seeding on the upper channel **helps saving cells** and makes possible to **concentrate them**. This will allow to create a **cell monolayer** along the channel easily/quickly.

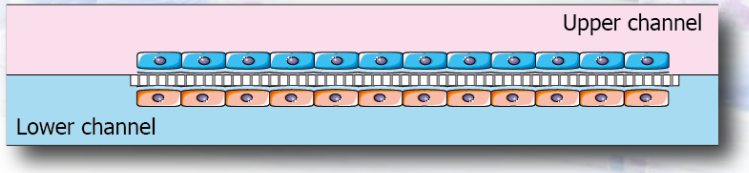
6. Add **PBS or water** to the **evaporation reservoirs** and incubate until the cells are completely attached (time may vary depending on the cell type).

7. Add more **medium to the reservoirs**, **cover and incubate** until reaching confluence.

8. Refresh medium according to cellular requirements. We recommend to **repeat this process** every one or two days.

Important: Be aware **not to remove the medium inside of the channel** during medium refreshing. This will prevent you to **avoid** the entrance of **air bubbles**. Thus, when removing the solution, **place the tip of the pipette away from the pinhole** to avoid emptying the channel.

For optimal seeding inside the channel, we recommend a constant renewal of the medium once the cells have adhered to the surface of the device (from two to six hours after seeding).



FLOW SET UP

Before setting the flow up:

- **Sterilize and prewarm** the tubes and the fluidic elements overnight at 37°C.
- Set the system in a **laminar flow cabinet**.
- **Cells must be well adhered** to the surface before mounting the perfusion system.
- The channels and inlet/outlet wells should **never be depleted of culture medium**.
- Both inlets and outlets are designed to be for **screw connectors (1/4" - 28).**

For more information regarding connecting a microfluidic flow system check our website.

Connecting a perfusion system

9. Remove the medium from the reservoirs.

10. Screw the **outlet connector** and ensure that the tube is perfectly fixed. **Remove the displaced medium** from the reservoir.

11. Prime the tube that will **connect to the inlet** before assembling. This will **prevent air bubbles** from entering the device.

12. Screw the inlet connector and remove the displaced medium from the reservoirs.

13. Once the system is closed, **switch the flow on**.

Check that there are **no leaks** in the system by leaving the pump **running for a couple of minutes** before placing the device in the incubator. Medium reservoirs will be filled up if the connections are not completely closed.

Important: Note that the channel should never dry up. Take incubation times into account and use the evaporation reservoirs as long as possible.

See youtube video:
Be-Doubleflow: Cell culture

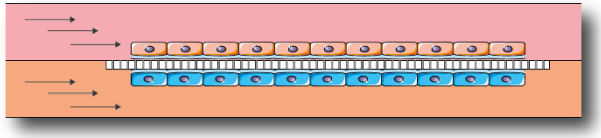
BE-DOUBLEFLOW

CULTURE MODELS

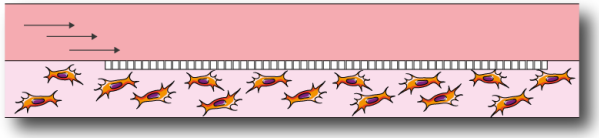
Depending on the assay, either **monoculture or coculture can be performed**. Find below several of the **most frequently used culture models** on the BE-Doubleflow:

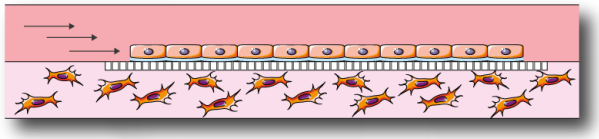
Seeding underneath the membrane

1) Seed a monolayer over and/or below the membrane with different cell types.

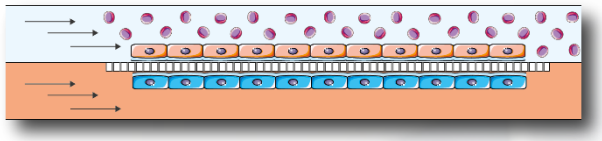


2) For more realistic assays it is possible to **add a hydrogel for 3D cell culture** or **combine 2D and 3D.**





3) Perfuse immune system cells, CTC´s or bacteria and explore their interaction with the culture.



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