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Protocol

Vena8 Endothelial+™ Biochip

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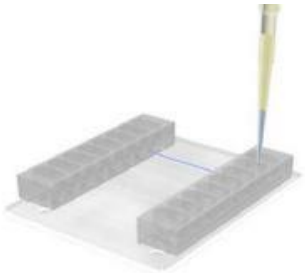
Vena8 Endothelial+ Biochip, Protocol #1: coating and cell seeding in Vena8 Endothelial+ biochips



Step 1:
Cellix Vena8 Endothelial+ biochip is coated using a standard yellow tip pipette, by dispensing approximately 12 µL of protein (e.g. fibronectin, 100 µg/mL) into each microchannel. Note the excess of liquid on the entrance and exit ports.

Step 2:
The Vena8 Endothelial+ biochip is then placed in a humidified box which remains open for 1–1.5 hours in the CO_2 incubator. Alternatively, the biochip may be placed at 4°C for overnight coating.

Step 3:
After the incubation period, add 5 µL of 1.5 x 10^6 /100 µL (≅ 15 x 10^6/mL)of endothelial cells gently into each channel.
Note: concentration specified is for primary HUVEC.
The biochip is kept in the CO_2 incubator for 15–20 minutes for the cells to adhere. Observe the biochip under a microscope and top up all the reservoirs with 50 µL of media. Keep the biochip for 1.5–2 hrs in the CO_2 incubator.

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Vena8 Endothelial+ Biochip Protocol #2: executing cell rolling, adhesion and migration assays under shear flow with Vena8 Endothelial+ biochips (manual version — not with VenaFlux platform)

Step 1:
Suspension cells (e.g. T cells, monocytes, platelets) are re-suspended in culture medium at an appropriate concentration (typically 2–5 x 10^6/mL) in an Eppendorf tube.

Step 2:
Using the Cellix Mirus Evo nanopump or the ExiGo pump, 10 µL of media is dispensed from pump output cable. Following this, the output cable is inserted into a specified channel on the Vena8 Endothelial+ biochip.

Step 3:
Then using the Cellix Mirus Evo nanopump or the ExiGo pump, 40 µL of the media is injected through the channel at a shear stress of 40 dynes/cm^2. This is done to wash the channel of cell debris. The waste is aspirated from the microwell of Vena8 Endothelial+ biochip with a pipette.

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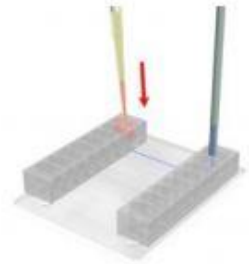
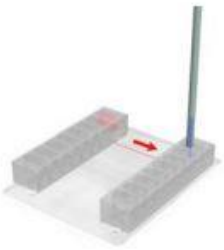
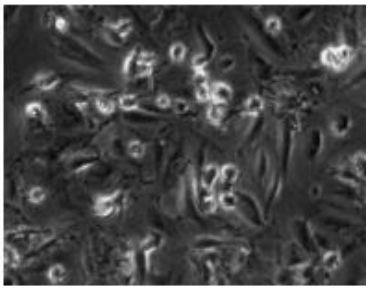


Step 4:
Cell sample is placed into the microwell of this channel on the Vena8 Endothelial+ biochip.



Step 5:
Cells are introduced into the channel, by specifying the desired shear stress on the FlowAssay software. The flow rate will be automatically calculated.



Step 6:
At each shear stress value, it is recommended that images of 3–5 fields of view of cell rolling and adhesion are acquired along the length of the channel.