

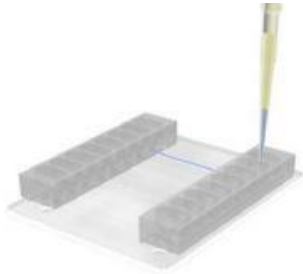


## Protocol

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Vena8 Endothelial+™ Biochip

## 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  $\mu\text{L}$  of protein (e.g. fibronectin, 100  $\mu\text{g}/\text{mL}$ ) 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  $\text{CO}_2$  incubator. Alternatively, the biochip may be placed at  $4^\circ\text{C}$  for overnight coating.



### Step 3:

After the incubation period, add 5  $\mu\text{L}$  of  $1.5 \times 10^6 / 100 \mu\text{L}$  ( $\cong 15 \times 10^6 / \text{mL}$ ) of endothelial cells gently into each channel.

**Note:** concentration specified is for primary HUVEC.

The biochip is kept in the  $\text{CO}_2$  incubator for 15–20 minutes for the cells to adhere. Observe the biochip under a microscope and top up all the reservoirs with 50  $\mu\text{L}$  of media. Keep the biochip for 1.5–2 hrs in the  $\text{CO}_2$  incubator.

## 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:

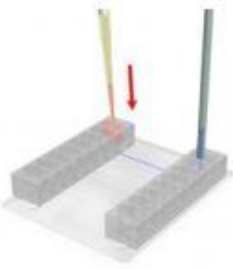
Suspension cells (e.g. T cells, monocytes, platelets) are re-suspended in culture medium at an appropriate concentration (typically  $2-5 \times 10^6/\text{mL}$ ) in an Eppendorf tube.

### Step 2:

Using the Cellix Mirus Evo nanopump or the ExiGo pump, 10  $\mu\text{L}$  of media is dispensed from pump output cable. Following this, the output cable is inserted into a specified channel on the Vena8 Endothelial+ biochip.

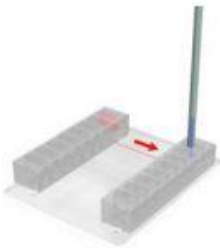
### Step 3:

Then using the Cellix Mirus Evo nanopump or the ExiGo pump, 40  $\mu\text{L}$  of the media is injected through the channel at a shear stress of 40 dynes/cm<sup>2</sup>. This is done to wash the channel of cell debris. The waste is aspirated from the microwell of Vena8 Endothelial+ biochip with a pipette.



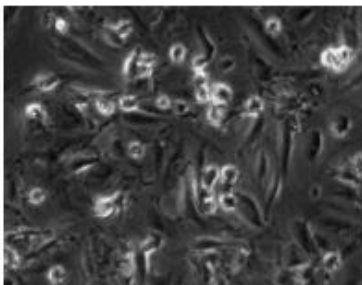
**Step 4:**

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.