

## Vena8 Endothelial+™ Biochip Protocol

### Protocol for Coating and Cell seeding in Vena8 Endothelial+™ Biochips

#### Step 1



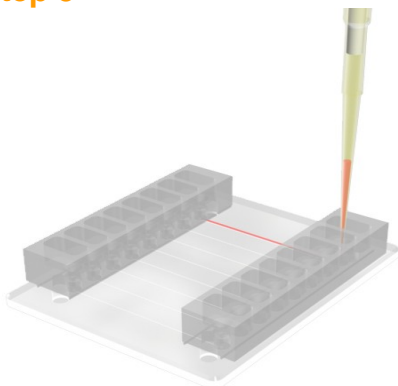
Cellix **Vena8 Endothelial+™** biochips are kept under UV for 20 minutes and coated using a standard yellow tip pipette, by dispensing approximately 12  $\mu\text{L}$  of protein (e.g. laminin) into each microchannel. Note the excess of liquid on the entrance and exit ports.

#### 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 approximately 5  $\mu\text{L}$  of  $1.5 \times 10^6$  per 100 $\mu\text{L}$  of endothelial cells gently into each channel.

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

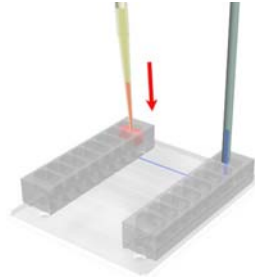
The biochip is kept in the  $\text{CO}_2$  incubator for 15 – 20 minutes. Observe the biochip under microscope and top up all the reservoirs with 40 $\mu\text{L}$  of media. Keep the biochip for 1.5 - 2 hrs in the  $\text{CO}_2$  incubator.

## Protocol for 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.



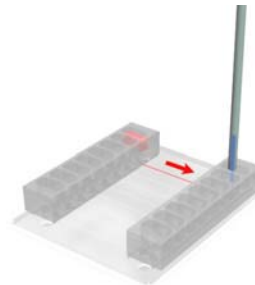
### Step 4

Cell sample is placed into the microwell of this channel on the **Vena8 Endothelial+™** biochip.



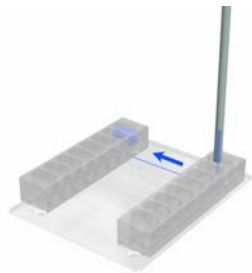
### Step 2

Using the Cellix **Mirus Nanopump™**, 1.5µl of media is dispensed from the Nanopump connector cable. Following this the Nanopump connector cable is inserted into a specified channel on the **Vena8 Endothelial+™** biochip.



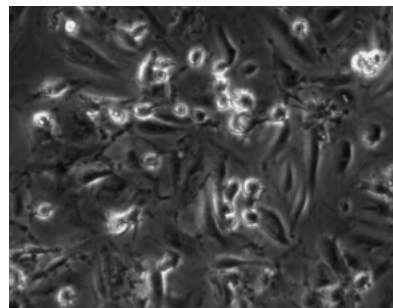
### Step 5

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



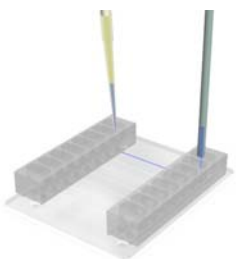
### Step 3

Then using the Cellix **Mirus Nanopump™**, 40 µL 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.



### Step 6

At each particular 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.



The waste is aspirated from the microwell of **Vena8 Endothelial+™** biochip with a pipette.