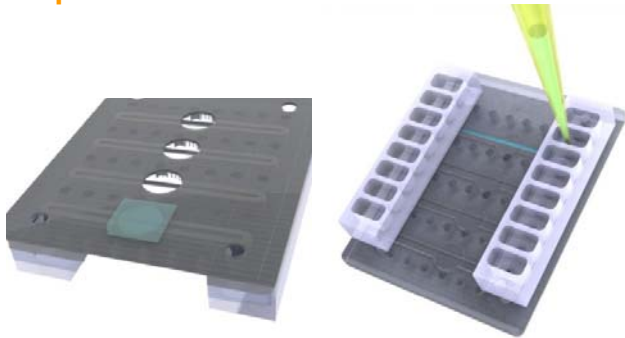


## VenaT4 Biochip Protocol

### Protocol for Coating VenaT4 Biochips

#### Step 1



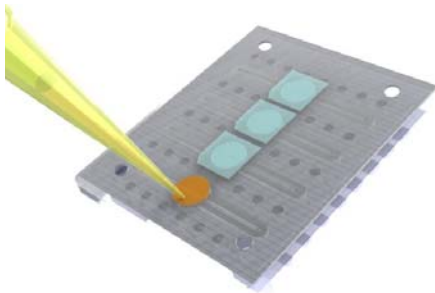
The **VenaT4** biochip micro-wells are sealed with a thin-film strip. The microchannels of the VenaT4 biochip are coated using a standard yellow tip pipette by dispensing approximately 50 $\mu$ L of protein (e.g. rhICAM) into each microchannel. Note the excess of liquid on the entrance and exit ports.

#### Step 2



Place the **VenaT4** biochip in a humidified box and keep incubated at 4°C for overnight coating.

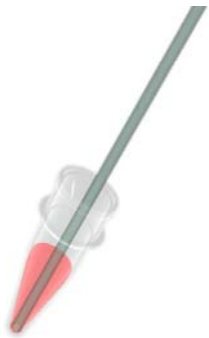
#### Step 3



After the incubation period, turn the biochip upside-down and remove the thin-film strips. Again using a standard yellow tip pipette, add approximately 30 $\mu$ L of Type I Bovine collagen gel solution with chemoattractant into the wells. Place the biochip into a humidified box kept in the CO<sub>2</sub> incubator for 15 – 20 minutes at 37°C.

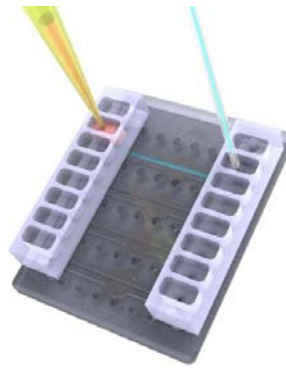
Once gel solidifies, re-seal the micro-wells with thin-film strips. The biochip is now ready to run the assay.

## Protocol for Transendothelial Migration Assays under Shear Flow with VenaT4 Biochips (Single Channel Version)



### Step 1

Suspension cells (e.g. T cells), are re-suspended in culture medium at a suitable concentration (typically  $5 \times 10^6/\text{mL}$ ) in an eppendorf. Cells are stained with appropriate dye.



### Step 4

Cell sample is placed into the micro-well of this channel on the **VenaT4** biochip.



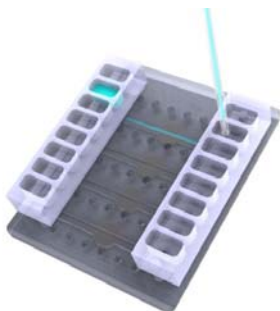
### Step 2

Using the Cellix **Mirus Nanopump™**, 30 $\mu\text{L}$  of media is dispensed from the Nanopump connector cable. Following this the Nanopump connector cable is inserted into a specified channel on the **VenaT4** biochip.



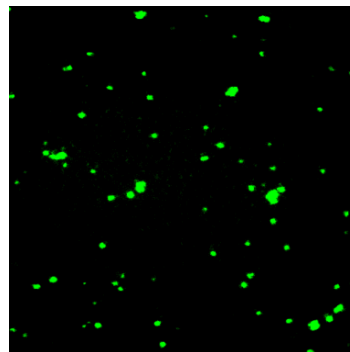
### Step 5

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



### Step 3

Then using the Cellix **Mirus Nanopump™**, 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. The waste is aspirated from the micro-well of **VenaT4** biochip with a pipette.



### Step 6

Time-lapse fluorescent images are recorded as the microscope objective is positioned over the micro-well. The rate of image capture is 6 frames per minute for 30 minutes.