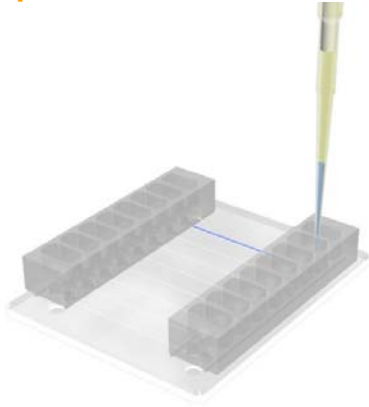


Vena8™ / Vena8 Fluoro+™ Biochip Protocols

Protocol for Coating Vena8™ / Vena8 Fluoro+™ Biochips

Step 1



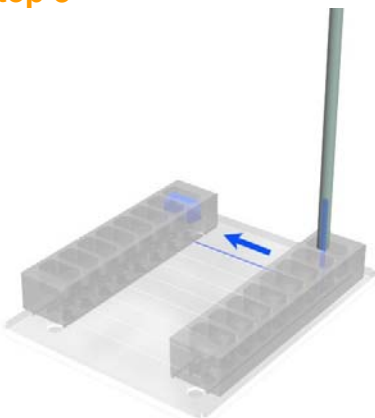
Cellix Vena8™ / Vena8 Fluoro+™ biochips are coated using a standard yellow tip pipette, by dispensing approximately 10 μ L of protein into each microchannel. Note the excess of liquid on the entrance and exit ports.

Step 2



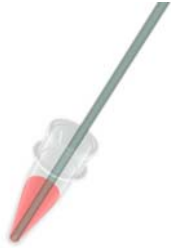
The Vena8™ / Vena8 Fluoro+™ biochip is then placed in a humidified box and sealed for 2 hours at room temperature or, alternatively, overnight at 4°C

Step 3



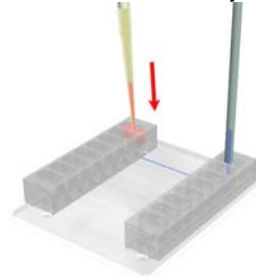
After the incubation period, add 10 μ L of 0.1% BSA into each channel to ensure specificity of binding during the adhesion assay. The biochip is kept in the humidified box for a further 30 minutes.

Protocol for Executing Cell Rolling, Adhesion and Migration Assays under Shear Flow with Vena8™ / Vena8 Fluoro+™ Biochips (Manual Version – not with VenaFlux Platform)



Step 1

Suspension cells (e.g. T cells, neutrophils, eosinophils, platelets) are re-suspended in culture medium at an appropriate concentration (typically 2-5 x 10⁶/mL) in an eppendorf.



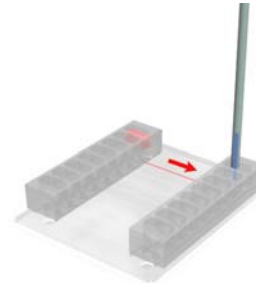
Step 5

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



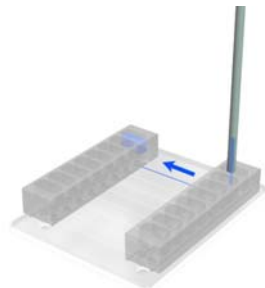
Step 2

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™ / Vena8 Fluoro+™** biochip.



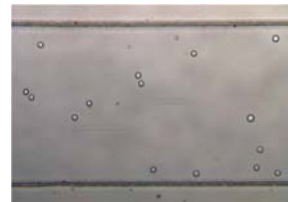
Step 6

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



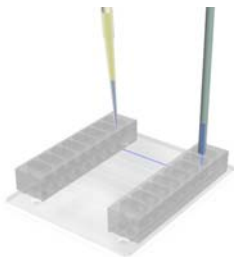
Step 3

Then using the Cellix **Mirus Nanopump™**, 40 µL of the media is injected through the channel at a shear stress of 40 dynes/cm². This is done to wash the biochip of excess ligand and BSA used during coating. The waste comes out on the other side of the biochip and is collected inside the biochip microwell.



Step 7

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.



Step 4

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