



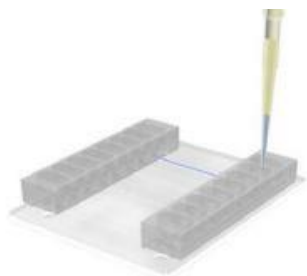
## Protocol

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### Vena8 Fluoro+™ Biochip

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## Vena8 Fluoro+ Biochip, Protocol #1: coating Vena8 Fluoro+ biochips

**Step 1:**

Cellix Vena8 Fluoro+ biochip is coated using a standard yellow tip pipette, by dispensing approximately 10  $\mu$ L of protein (e.g. VCAM, ICAM, etc.) into each microchannel. Note the excess of liquid on the entrance and exit ports.

**Step 2:**

The 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  $\mu$ 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.

Vena8 Fluoro+ Biochip Protocol #2: executing cell rolling, adhesion and migration assays under shear flow with 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  $2-5 \times 10^6$ /mL) in an Eppendorf tube.



**Step 2:**

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



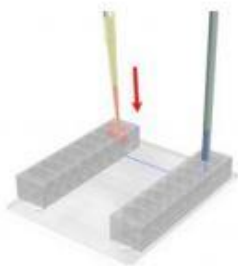
**Step 3:**

Then using the Cellix Mirus Evo nanopump, or the ExiGo pump, 40  $\mu$ 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 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.



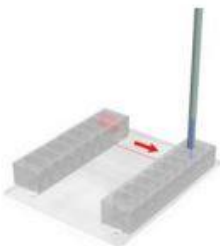
**Step 4:**

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



**Step 5:**

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



**Step 6:**

Cells are introduced into the channel, by specifying the desired shear stress using VenaFlowAssay software or SmartFlo. The flow rate will be automatically calculated.



**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.

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