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# Protocol

## Thrombosis Assays with Mirus Evo™ or ExiGo™ Pump

**Cellix Ltd.**
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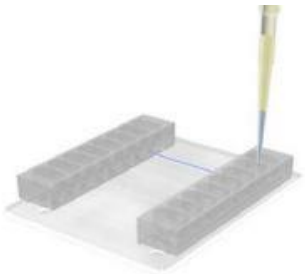
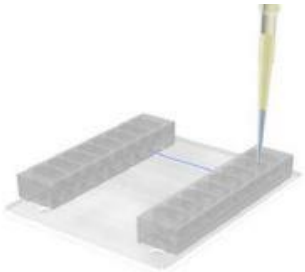
# Thrombosis Assays with Mirus Evo Nanopump or ExiGo Pump Protocol: to coat channels of Vena8 Fluoro+ biochip with collagen and to perform thrombosis experiment using Mirus Evo or ExiGo pump



**Step 1:**
Cellix's Vena8 Flouro+ biochip is coated using a standard yellow tip pipette, by dispensing approximately 12 µL of fibrillar collagen (200 µg/mL, NYCOMED 1130630) into each channel. Note the excess of liquid on the entrance and exit ports.

**Step 2:**
The Vena8 Fluoro+ biochip is then placed in a humidified box, which should be placed at 4°C for overnight coating.



**Step 3:**
After the incubation period, add 10 µL of 10% BSA into each channel to ensure specificity of binding during the adhesion assay. The biochip is kept in the humidified box for a further 15 minutes at room temperature.

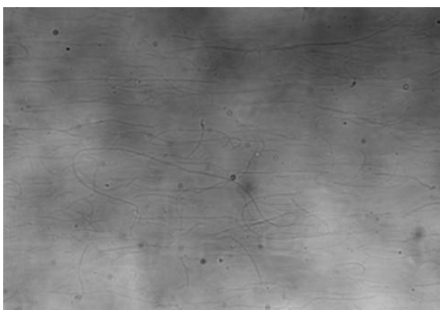


Image: acquired after overnight coating of collagen (200 µg/mL) in the channels of Vena8 Fluoro+ biochip (20X magnification).

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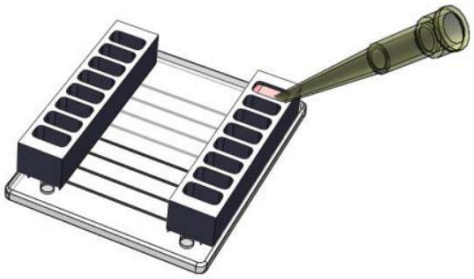

**Step 1:**
Add 40 µL of media/buffer into the reservoir of the biochip before connecting the out cable from the pump.

**Step 2:**
Connect one end of thrombosis cable (left image) to the output cable of Mirus Evo nanopump/ExiGo pump and other end to the biochip inlet cable (right image).

**Step 3:**
Using the Cellix Mirus Evo nanopump or the ExiGo pump, 10 µL of media is dispensed from the pump output cable. Following this, the output cable is inserted into a specified channel on the Vena8 Fluoro+ biochip.

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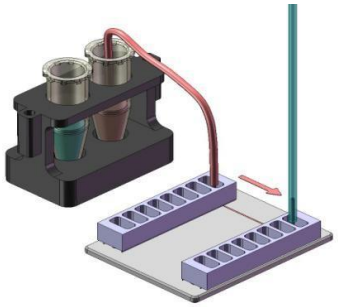
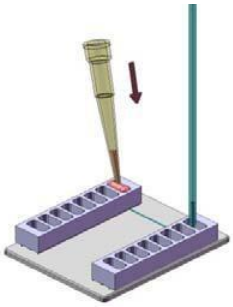
**Step 4:**

Then using the Cellix Mirus Evo nanopump or the ExiGo™ pump, 40 µL of the media is injected through the channel at a shear stress of 40 dynes/cm$^2$. This is done to wash the biochip of excess collagen and BSA used during coating. The waste comes out on the other side of the biochip and is collected in an Eppendorf tube.



**Step 5:**

Sample (e.g. whole blood, PRP, etc.) is introduced into the channel, by specifying the desired shear stress using VenaFlowAssay software or SmartFlo. The flow rate will be automatically calculated.



**Step 6:**

Disconnect the tubing from the blood sample tube after infusion of sample. Add buffer, e.g. PBS (100 µL) into the reservoir using a pipette and pull (aspirate) the buffer at appropriate shear stress Using Mirus Evo nanopump or the ExiGo pump. This is to wash whole blood from the channel.