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Protocol

VenaT4™ Biochip

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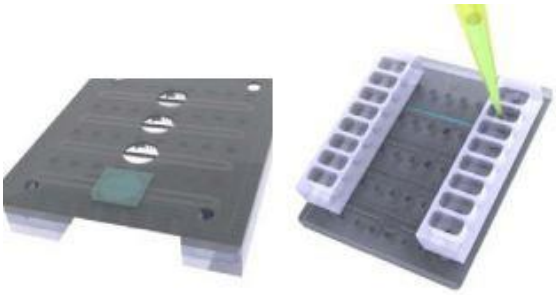
VenaT4 Biochip, Protocol #1: coating VenaT4 biochips

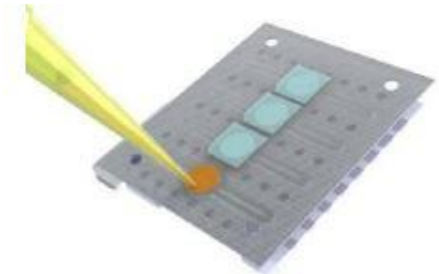


Step 1

The VenaT4 biochip microwells 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 µL of protein (e.g. rhICAM) into each microchannel. Note the excess of liquid on the entrance and exit ports.

Step 2

The VenaT4 biochip is then placed in a humidified box and 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 µL of Type I Bovine collagen gel solution with chemoattractant into the wells.
Place the biochip into a humidified box kept in the CO_2 incubator for 15–20 minutes at 37°C. Once gel solidifies, re-seal the microwells with thin-film strips. The biochip is now ready to run the assay.

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VenaT4 Biochip Protocol #2: trans endothelial 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 an appropriate concentration (typically 5 x 10^6/mL) in an Eppendorf tube. Cells are stained with a suitable dye.

Step 2:
Using the Cellix Mirus Evo nanopump or the ExiGo pump, 30 µL of media is dispensed from pump output cable. Following this, the output cable is inserted into a specified channel on the VenaT4 biochip.

Step 3:
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 channel. The waste is aspirated from the microwell of VenaT4 biochip with a pipette.

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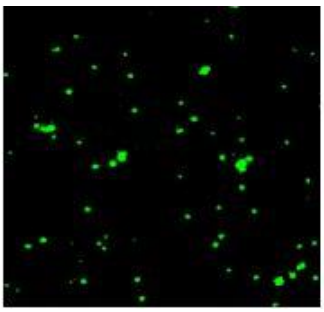
Step 4:
Cell sample is placed into the microwell of this channel on the VenaT4 biochip.

Step 5:
Cells are introduced into the channel, by specifying the desired shear stress using VenaFlux Assay software or SmartFlo application. The flow rate will be automatically calculated.



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