

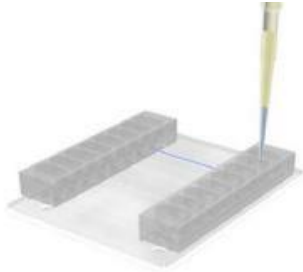


Protocol

Kima™ Pump

Kima Pump Perfusion Protocol: coating and cell seeding in Vena8 Endothelial+ biochips and perfusion using Kima pump

NOTE: Kima pump is only for exchange of culture media to feed the cells (i.e. pulses of fluid / pulsatile flow) rather than a continuous flow.



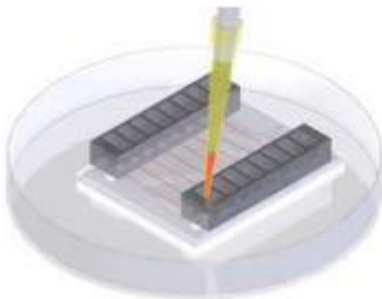
Step 1:

Cellix's Vena8 Endothelial+ biochips are coated using a standard pipette tip. Dispense $\sim 12 \mu\text{L}$ of protein (e.g. fibronectin) into each microchannel. Note the excess of liquid on the entrance and exit ports.



Step 2:

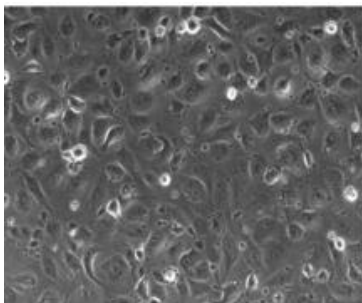
The Vena8 Endothelial+ biochip is then placed in a humidified sterile petri dish, which should be placed at 4°C for overnight coating.



Step 3:

(a) After the incubation period, add $5 \mu\text{L}$ of $1.5 \times 10^6 / 100 \mu\text{L}$ ($\cong 15 \times 10^6/\text{mL}$) of endothelial cells gently into each channel. ***Note:** concentration specified is for primary HUVEC.

(b) The biochip is kept in a sterile petri dish and in the CO_2 incubator for 15–20 minutes. Observe the biochip under microscope and top up all the reservoirs with $50 \mu\text{L}$ of media. Keep the biochip for 1.5–2 hours in the CO_2 incubator.



HUVEC primary cells seeded in Vena8 Endothelial+ biochip microchannel: 1.5 hrs post-incubation.



Step 4:

Wash Kima pump with 70% ethanol and then with sterile media using a 5 mL sterile syringe in the biosafety hood.



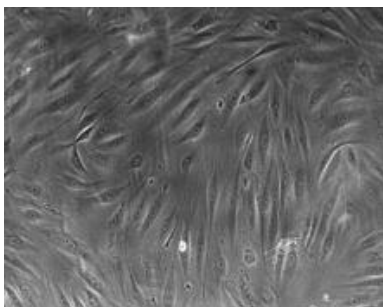
Step 5:

Connect tubing from media bottle to the inlet port of the pump. Connect tubing from outlet port of the pump (8-way cable with pins) to a sterile petri dish. Wash the pump using iKima app with media for 3 minutes.



Step 6:

(a) Take the biochip from the incubator and place in the biosafety hood. Before connecting to the biochip, start perfusion using iKima app — typically 2 minutes perfusion, followed by 15–20 minutes pause. When media droplets form at the pins, gently connect the 8-way cable to the biochip to avoid air bubbles.



HUVEC primary cells seeded in Vena8 Endothelial+ biochip microchannel: 72 hrs post-perfusion with Kima pump.

(b) Connect the outlet pins to the biochip which is connected to a discard bottle or to the same media bottle for recirculation of the same media. This is done in the biosafety hood. Once connected, transfer Kima platform to a CO₂ incubator.

Note: all tubing and bottles must be autoclaved prior to the experiment.