## Prepare chip

Surface activation
Coat both channels with ER-1
Activate by UV treatment (20 min)



Coat chips with extracellular matrix
Top Channel – laminin 50 ng/mL
Bottom channel – collagen IV 320
ng/mL: fibronectin 80 ng/mL
Incubate overnight (37°C)

#### Seed cells

### **Top Channel**

Dissociate EZ-sphere
Seed cells (1-6 x 10<sup>6</sup> cells/mL)
Incubate overnight (37°C)
Wash cells that did not attach



#### **Bottom Channel**

Dissociate iBMECs
Seed cells (14-20 x 10<sup>6</sup> cells/mL)
Incubate chip upside-down (3 h, 37 °C)
Flip chip to upright and incubate 3 h

# Laminar flow 48 h post seeding

Insert chips to culture module
Prime pods (5 min)
Insert chip to pod

Run Regulate cycle (2 h)



Initiate flow Flow rate 30-1000 μL/h