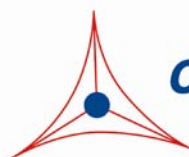

Product Manual

CytoSelect™ 8-Channel Endothelial Microfluidic Biochips

Catalog Number

CBA-004	2 chips
CBA-004-5	10 chips

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

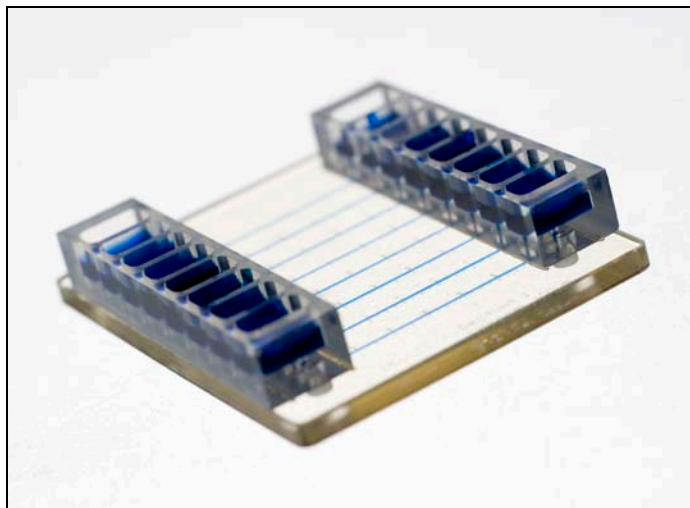
Shear stresses are continually created in the body by many physiological processes including the flow of blood through arteries and veins, the movement of saliva over teeth and mucus membranes, and even mucus movement inside the airways of the lungs. These *in vivo* shear stresses create forces that can affect the activation state of cell surface receptors. Traditional *in vitro* cell-based assays for measuring cell adhesion, cell rolling and cell migration do not take these shear stress forces into account.

CytoSelect™ Biochips provide a microfluidic environment that closely mimics *in vivo* shear stresses. CytoSelect™ Biochips are useful for measuring cell adhesion and related processes while providing more physiologically relevant data than traditional static assays. The Biochips are versatile and are available in a variety of formats for a wide variety of applications:

- Cell adhesion, rolling and migration
- Cell proliferation
- Thrombosis
- Cell-cell interactions
- Endothelial / epithelial cell monolayer culture
- Single cell / platelet analysis
- Immunostaining

Assay Principle

The CytoSelect™ 8-Channel Endothelial Biochip is a self-contained unit containing 8 channels with an inlet/outlet port at both ends of each channel. The channels of the 8-Channel Endothelial Biochip are first coated with one or more ligands, e.g. adhesion molecules such as collagen, fibrinogen, by dispensing the ligand into one of the ports. After an incubation period, endothelial cells such as HUVEC are added. Then the cells to be assayed are added using a microfluidic syringe pump at a specified flow rate that mimics *in vivo* shear stresses. Cell adhesion may be visualized by brightfield or phase contrast microscopy.



Kit Components

CytoSelect™ Endothelial Microfluidic Biochip (Part No. 100401): Two 8-channel chips

Related Products

1. CBA-003: CytoSelect™ 8-Channel ECM Microfluidic Biochips
2. CBA-050: CytoSelect™ 48-Well Cell Adhesion Assay (Fibronectin-Coated, Colorimetric Format)
3. CBA-053: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen I-Coated, Fluorometric Format)
4. CBA-056: CytoSelect™ 48-Well Cell Adhesion Assay (Laminin-Coated, Colorimetric Format)
5. CBA-059: CytoSelect™ 48-Well Cell Adhesion Assay (Fibrinogen-Coated, Fluorometric Format)
6. CBA-060: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen IV-Coated, Colorimetric Format)
7. CBA-071: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Fluorometric Format)

Materials Not Supplied

1. Suspension cells
2. Cell culture medium
3. Cell culture incubator (37°C, 5% CO₂ atmosphere)
4. Humidified box
5. Single channel micropipette with disposable tips
6. Syringe pump
7. Bright field or phase contrast microscope

Storage

Store Biochips at room temperature.

Preparation of Samples

Resuspend cells in culture medium at a concentration of $2-5 \times 10^6$ cells/mL. Suitable samples for the CytoSelect™ 8-Channel Endothelial Biochip include the following:

- T-cells, primary and established cell lines (e.g. HUT 78)
- Monocytes, primary and established cell lines (e.g. THP-1)
- Eosinophils
- Neutrophils
- Platelets
- PBMCs
- Whole blood

Assay Protocol

1. Coat each channel of the Biochip by dispensing 12 μL of desired protein (e.g. laminin) into the proximal port at one end of each channel. Excess liquid will collect in the ports at both ends.



2. Place the Biochip into a humidified box and place box with lid open in a CO_2 incubator. Incubate for 60-90 minutes at 37°C .



3. Add 5 μL of endothelial cells gently into each channel.

Note: For HUVEC cells use a concentration of 1.5×10^7 cells/mL.



4. Place the Biochip back into the open humidified box in the CO_2 incubator. Incubate for 15-20 min.

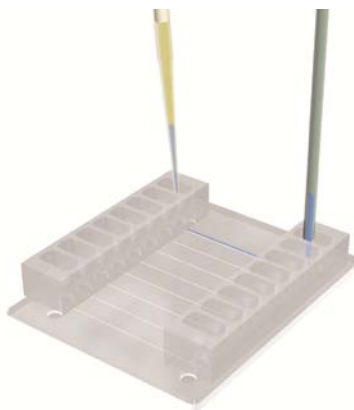
5. Observe the Biochip under a microscope and top up each reservoir with 40 μL of media.



6. Place the Biochip back into the open humidified box in the CO_2 incubator. Incubate for 60-90 min.
7. Set up a syringe pump to dispense culture media. Dispense a small amount of media (1-2 μL) from the connector cable into a waste container, and then insert the connector cable into the proximal port of the Biochip.



8. Dispense 40 μL of media at a shear stress of 40 dynes/cm^2 , a shear rate of 4000 s^{-1} , or a flow rate of $160 \mu\text{L/min}$. This washes the Biochip of excess cell debris. Repeat for each channel.
9. Aspirate media collected in the port at the opposite end of each channel.



10. Using a standard micropipette, add 10 μL to 100 μL of cell suspension to the opposite port of each channel.



11. With the connector cable of the syringe pump in the proximal port, apply the desired shear stress, shear rate, or flow rate to the channel. Cells will move from the opposite port through the channel. Suggested pump settings may be found in Table 1.



Sample Type	Shear Stress Range (dynes/cm ²)	Shear Rate Range (s ⁻¹)	Flow Rate Range ($\mu\text{L}/\text{min}$)
Cell Suspensions	0.5 – 10	50 – 1000	2 – 40
Whole Blood	2.25 – 450	50 – 10,000	2 – 400

Table 1. Recommended Shear Stress, Shear Rate or Flow Rate to Mimic *In Vivo* Forces.

12. Using brightfield, phase contrast, fluorescence or confocal microscopy, acquire 3 to 5 images along the length of each channel using a magnification of 60X to 100X.

Example of Results

The following figures demonstrate typical results with the CytoSelect™ 8-Channel Biochip. One should use the data below for reference only. This data should not be used to interpret actual results.

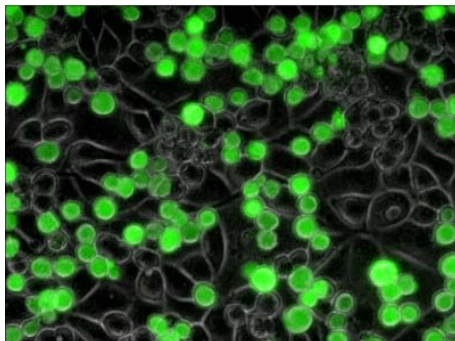


Figure 1. MDA-GFP Adhesion on HUVEC Cells. Biochip was coated with TNF-treated HUVEC cell line. Cells were applied to the Biochip at a shear stress rate of 0.5 dyne/cm².

Warranty

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