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Product Manual

# CytoSelect™ 48-Well Cell Adhesion Assay (Collagen IV-Coated, Colorimetric Format)

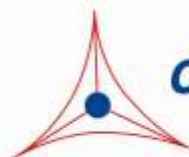
Catalog Number

CBA-060

48 assays

**FOR RESEARCH USE ONLY**  
Not for use in diagnostic procedures

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*Creating Solutions for Life Science Research*

## **Introduction**

Cell adhesion is a complex process involved in embryogenesis, migration/invasion, tissue remodeling, and wound healing. To perform these processes, cells adhere to extracellular matrix components (via adhesion receptors), forming complexes with components of the cytoskeleton that ultimately affect cell motility, differentiation, proliferation, and survival. The Cell Biolabs CytoSelect™ Cell Adhesion Assay Kit provides a rapid, quantitative method for evaluating cell adhesion. The kit contains sufficient reagents for the evaluation of 48 samples (40 Human Collagen IV-coated wells, 8 BSA-coated wells).

## **Assay Principle**

The CytoSelect™ Cell Adhesion Assay Kit utilizes a Collagen IV-coated 48-well plate (see Adhesion Plate Layout below). First, cells are seeded onto the coated substrate, where the adherent cells are captured. Next, unbound cells are washed away, and the adherent cells are fixed/stained. Finally, the stain is extracted and quantified colorimetrically.

## **Related Products**

1. CBA-050: CytoSelect™ 48-Well Cell Adhesion Assay, (Fibronectin-Coated, Colorimetric Format)
2. CBA-051: CytoSelect™ 48-Well Cell Adhesion Assay (Fibronectin-Coated, Fluorometric Format)
3. CBA-052: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen I-Coated, Colorimetric Format)
4. CBA-053: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen I-Coated, Fluorometric Format)
5. CBA-056: CytoSelect™ 48-Well Cell Adhesion Assay (Laminin-Coated, Colorimetric Format)
6. CBA-057: CytoSelect™ 48-Well Cell Adhesion Assay (Laminin-Coated, Fluorometric Format)
7. CBA-058: CytoSelect™ 48-Well Cell Adhesion Assay (Fibrinogen-Coated, Colorimetric Format)
8. CBA-059: CytoSelect™ 48-Well Cell Adhesion Assay (Fibrinogen-Coated, Fluorometric Format)
9. CBA-061: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen IV-Coated, Fluorometric Format)
10. CBA-070: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Colorimetric Format)
11. CBA-071: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Fluorometric Format)

## **Kit Components**

1. Collagen Adhesion Plate (Part No. 106001): One 48-well plate containing 40 Human Collagen IV-coated wells and 8 BSA-coated wells (see layout below)
2. Cell Stain Solution (Part No. 11002): One Bottle – 10.0 mL
3. Extraction Solution (Part No. 11003): One Bottle – 10.0 mL

## **Adhesion Plate Layout**

The following layout indicates the location of wells coated with Collagen IV and those coated with BSA.

	1	2	3	4	5	6	7	8
A	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV
B	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV
C	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV
D	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV
E	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV	Collagen IV
F	BSA	BSA	BSA	BSA	BSA	BSA	BSA	BSA

### **Materials Not Supplied**

1. Cell culture medium
2. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM CaCl<sub>2</sub> and 2 mM MgCl<sub>2</sub>
3. Cell culture incubator (37°C, 5% CO<sub>2</sub> atmosphere)
4. 1X PBS containing 2 mM CaCl<sub>2</sub> and 2 mM MgCl<sub>2</sub>
5. Light microscope
6. 96-well microtiter plate
7. Microtiter plate reader

### **Storage**

Store all kit components at 4°C.

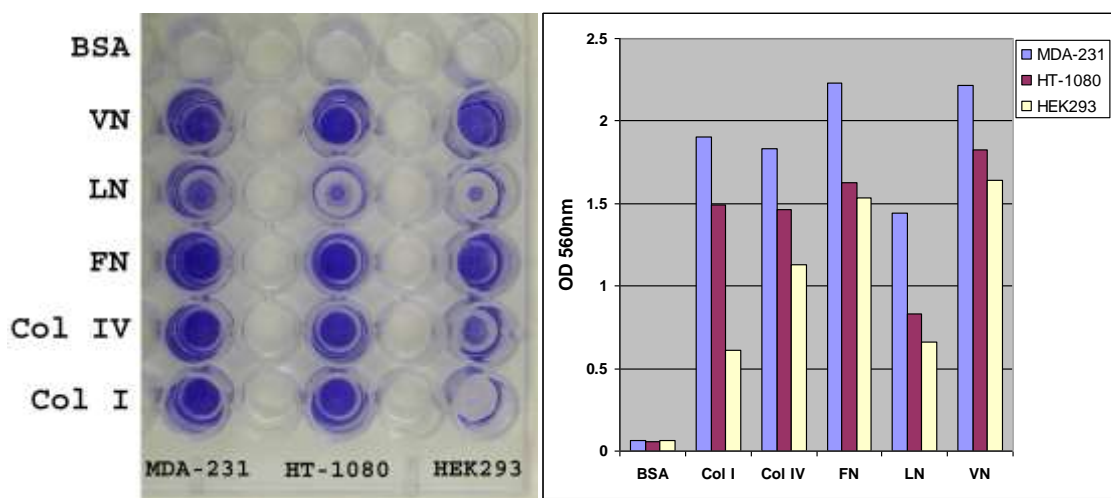
### **Assay Protocol**

1. Under sterile conditions, allow the Collagen IV Adhesion Plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing 0.1-1.0 x 10<sup>6</sup> cells/ml in serum free media. Agents that inhibit or stimulate cell adhesion can be added directly to the cell suspension.
3. Add 150 µL of the cell suspension to the inside of each well (BSA-coated wells are provided as a negative control).
4. Incubate for 30-90 min in a cell culture incubator.
5. **Carefully** discard or aspirate the media from each well (**Note: Do not allow wells to dry**). Gently wash each well 4-5 times with 250 µL PBS.

- Aspirate the PBS from each well and add 200  $\mu$ L of Cell Stain Solution. Incubate for 10 minutes at room temperature.
- Discard or aspirate the Cell Stain Solution from the wells. Gently wash each well 4-5 times with 500  $\mu$ L deionized water.
- Discard the final wash and let the wells air dry.
- Add 200  $\mu$ L of Extraction Solution per well, and then incubate 10 minutes on an orbital shaker.
- Transfer 150  $\mu$ L from each extracted sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

### **Example of Results**

The following figures demonstrate typical results with the CytoSelect™ 48-Well Cell Adhesion Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.



**Figure 1. ECM-mediated Cell Adhesion.** Serum starved cells were allowed to attach to ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were stained (left panel picture) and quantified at OD 560nm after extraction (right panel figure).

### **References**

- Hynes, R. O. (1992) *Cell* **69**, 11-25.
- Schwartz, M. A., Schaller, M. D. and Ginsberg, M. H. (1995) *Annu. Rev. Cell Dev. Biol.* **11**, 549-599.

### **Recent Product Citations**

- Tanoue, A. et al. (2021). Podocyte-specific Crb2 knockout mice develop focal segmental glomerulosclerosis. *Sci Rep.* **11**(1):20556. doi: 10.1038/s41598-021-00159-z.
- Geng, Y. et al. (2019). PD-L1 on invasive fibroblasts drives fibrosis in a humanized model of idiopathic pulmonary fibrosis. *JCI Insight.* **4**(6). pii: 125326. doi: 10.1172/jci.insight.125326.

- Miao, H. et al. (2008). Gene expression and functional studies of the optic nerve head astrocyte transcriptome from normal African Americans and Caucasian Americans donors. *PLoS One* **3(8)**:E2847.

### **Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

### **Contact Information**

Cell Biolabs, Inc.  
7758 Arjons Drive  
San Diego, CA 92126  
Worldwide: +1 858-271-6500  
USA Toll-Free: 1-888-CBL-0505  
E-mail: [tech@cellbiolabs.com](mailto:tech@cellbiolabs.com)  
[www.cellbiolabs.com](http://www.cellbiolabs.com)

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