
Product Manual

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

Catalog Number

CBA-052

48 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



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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 Bovine Collagen I-coated wells, 8 BSA-coated wells).

Assay Principle

The CytoSelect™ Cell Adhesion Assay Kit utilizes a Collagen I-coated 48-well plate (see Adhesion Plate Layout). 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-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-057: CytoSelect™ 48-Well Cell Adhesion Assay (Laminin-Coated, Fluorometric Format)
6. CBA-058: CytoSelect™ 48-Well Cell Adhesion Assay (Fibrinogen-Coated, Colorimetric Format)
7. CBA-059: CytoSelect™ 48-Well Cell Adhesion Assay (Fibrinogen-Coated, Fluorometric Format)
8. CBA-060: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen IV-Coated, Colorimetric 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. 105201): One 48-well plate containing 40 Bovine Collagen I-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 and those coated with BSA.

	1	2	3	4	5	6	7	8
A	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I
B	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I
C	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I
D	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I
E	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I	Collagen I
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 CaCl₂ and 2 mM MgCl₂
3. Cell culture incubator (37°C, 5% CO₂ atmosphere)
4. 1X PBS containing 2 mM CaCl₂ and 2 mM MgCl₂
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 I Adhesion Plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing 0.1-1.0 x 10⁶ 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 μ L PBS.
6. Aspirate the PBS from each well and add 200 μ L of Cell Stain Solution. Incubate for 10 minutes at room temperature.
7. Discard or aspirate the Cell Stain Solution from the wells. Gently wash each well 4-5 times with 500 μ L deionized water.
8. Discard the final wash and let the wells air dry.
9. Add 200 μ L of Extraction Solution per well, and then incubate 10 minutes on an orbital shaker.
10. Transfer 150 μ 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.

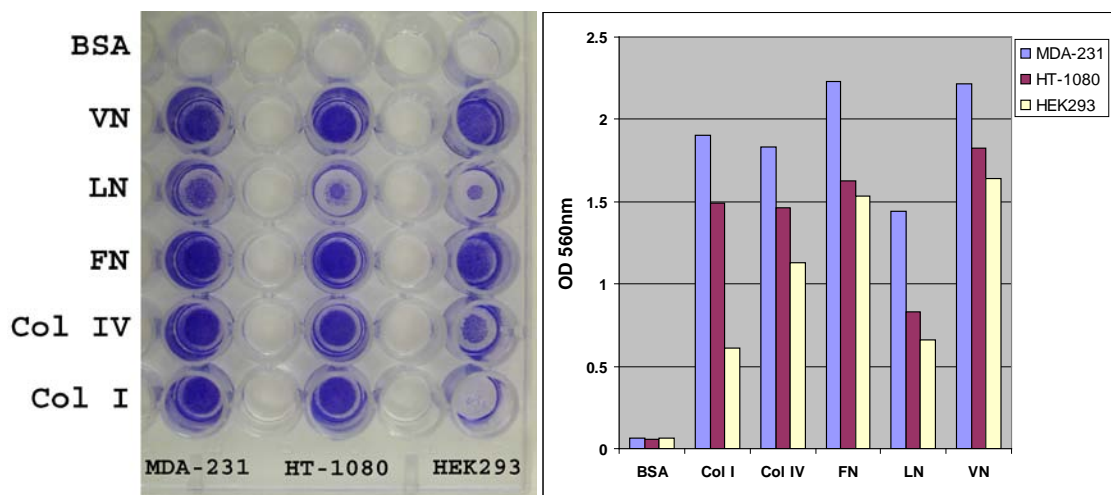


Figure 1. ECM-mediated Cell Adhesion. Serum starved cells were allowed to attach to ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were stained (left panel picture) and quantified at OD 560nm after extraction (right panel figure).

References

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

Recent Product Citations

1. Lee, J. H. et al. (2016). Fucoidan improves bioactivity and vasculogenic potential of mesenchymal stem cells in murine hind limb ischemia associated with chronic kidney disease. *J Mol Cell Cardiol.* **97**:169-179.
2. Wangpu, X. et al. (2016). Targeting the metastasis suppressor, n-myc downstream regulated gene-1, with novel Di-2-pyridylketone thiosemicarbazones: Suppression of tumor cell migration and cell-collagen adhesion by inhibiting focal adhesion kinase/paxillin signaling. *Mol Pharmacol.* **89**:521-540.
3. Aghajanian, H. et al. (2014). Semaphorin 3d and Semaphorin 3e direct endothelial motility through distinct molecular signaling pathways. *J Biol Chem.* **289**:17971-17979.

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