KAMIYA BIOMEDICAL COMPANY

Cell Adhesion Assay (ECM Array, Colorimetric)

For the rapid, quantitative evaluation of cell adhesion

Cat. No. KT-838

For Research Use Only. Not for use in diagnostic procedures.

PRODUCT INFORMATION

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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 Adhesion Assay Kit provides a rapid, quantitative method for evaluating cell adhesion. The kit contains sufficient reagents for the evaluation of 48 samples (40 ECM protein-coated wells, 8 BSA-coated wells).

PRINCIPLE
The Cell Adhesion Assay Kit utilizes an ECM protein-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.

COMPONENTS
1. ECM Adhesion Plate: One 48-well plate containing 40 ECM protein-coated wells and 8 BSA-coated wells (see layout below). FN, Collagen IV and Fibrinogen are from human, Laminin I is from Mouse and Collagen I is from Bovine.

2. Cell Stain Solution: One Bottle – 10.0 mL

3. Extraction Solution: One Bottle – 10.0 mL

ADHESION PLATE LAYOUT
The following layout indicates the location of wells coated with each ECM protein and those coated with BSA.

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

MATERIALS REQUIRED BUT NOT PROVIDED
1. Cell culture medium

2. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$

3. Cell culture incubator (37ºC, 5% CO_2 atmosphere)

4. 1X PBS containing 2 mM $CaCl_2$ and 2 mM $MgCl_2$

5. Light microscope

6. 96-well microtiter plate

7. Microtiter plate reader

STORAGE
Store all kit components at 4ºC.

ASSAY PROCEDURE
1. Under sterile conditions, allow the ECM Adhesion Plate to warm up at room temperature for 10 minutes.

2. Prepare a cell suspension containing 0.1-1.0 x 10^6 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.

6. Aspirate the PBS from each well and add 200 µL 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 µL deionized water.

8. Discard the final wash and let the wells air dry.

9. Add 200 µL of Extraction Solution per well, and then incubate 10 minutes on an orbital shaker.

10. Transfer 150 µL from each extracted sample to a 96-well microtiter plate and measure the OD 560 nm in a plate reader.

EXAMPLE OF RESULTS
The following figures demonstrate typical results with the 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.

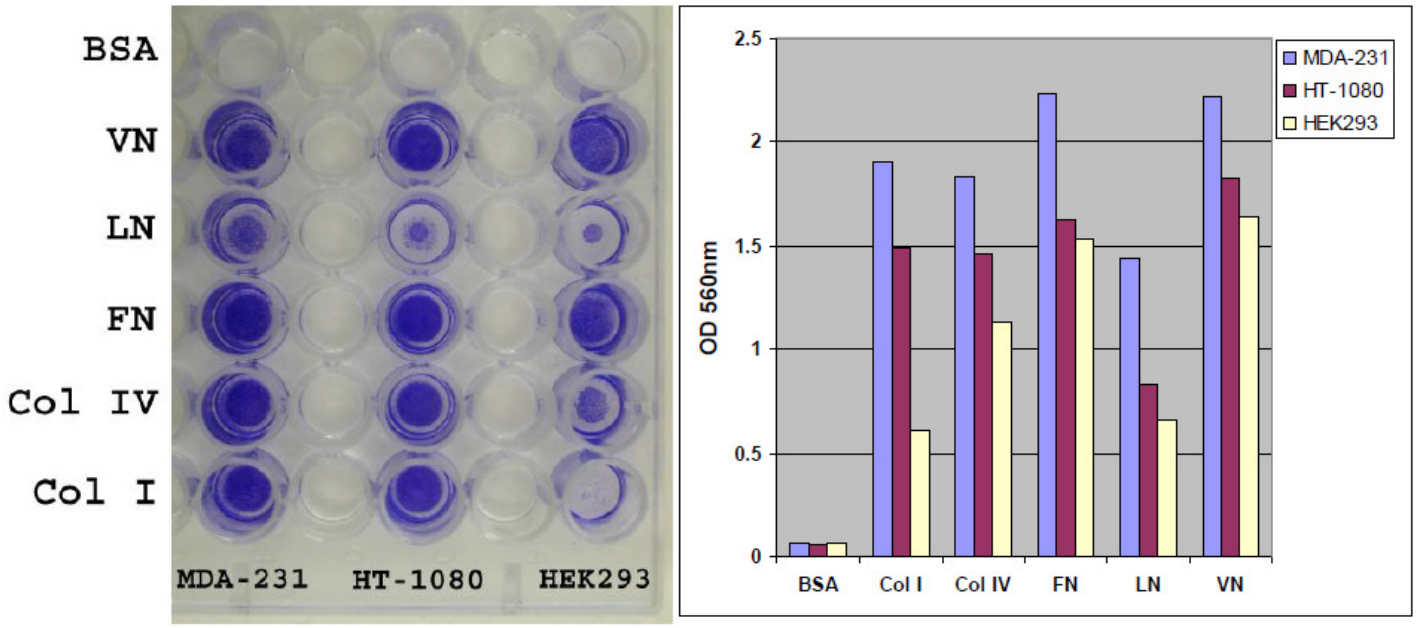

Figure 1. ECM-mediated Cell Adhesion. Serum starved cells were allowed to attach to ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were stained (left panel picture) and quantified at OD 560 nm after extraction (right panel figure).

FOR RESEARCH USE ONLY

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