Product Manual

CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Fluorometric Format)

Catalog Number

CBA-071	48 assays
CBA-071-5	5 x 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 ECM protein-coated wells, 8 BSA-coated wells).

Assay Principle

The CytoSelect™ Cell Adhesion Assay Kit utilizes an ECM protein-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 removed with consecutive washes. Finally, the adherent cells are lysed and subsequently detected with CyQuant® GR Dye.

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-070: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Colorimetric Format)

Kit Components (shipped at room temperature)

1. ECM Adhesion Plate (Part No. 107001): 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. 4X Lysis Buffer (Part No. 10404): One Bottle – 10.0 mL
3. CyQuant® GR Dye (Part No. 105101): One tube – 50 µL

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

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Materials Not Supplied

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 plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

Storage

Store all kit components at 4ºC.

Assay Protocol

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. Prepare sufficient 1X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:300 in Lysis Buffer (for example, add 4 µL dye to 300 µL of 4X Lysis Buffer and 900 µL of dH_2O).
7. Add 200 µL of 1X Lysis Buffer/CyQuant® GR dye solution to each well containing cells. Incubate 20 minutes at room temperature with shaking.
8. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480 nm/520 nm.

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.

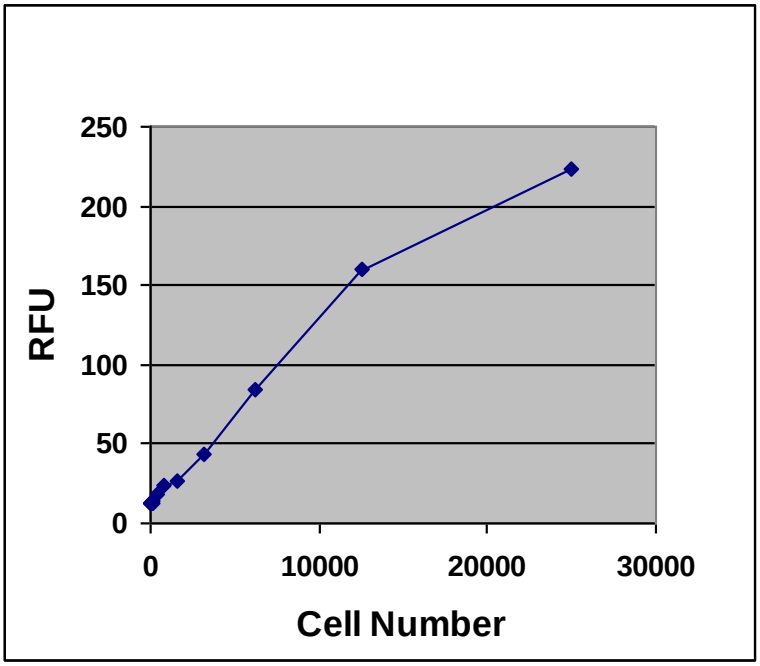

Figure 1: **Quantitation of Human HT-1080**. HT-1080 cells were titrated in 1X PBS, then subsequently lysed and detected with 1X Lysis Buffer/CyQuant® GR Dye (225 µL of cell suspension was mixed with 75 µL of 4X Lysis Buffer and 1 µL of CyQuant® GR dye).

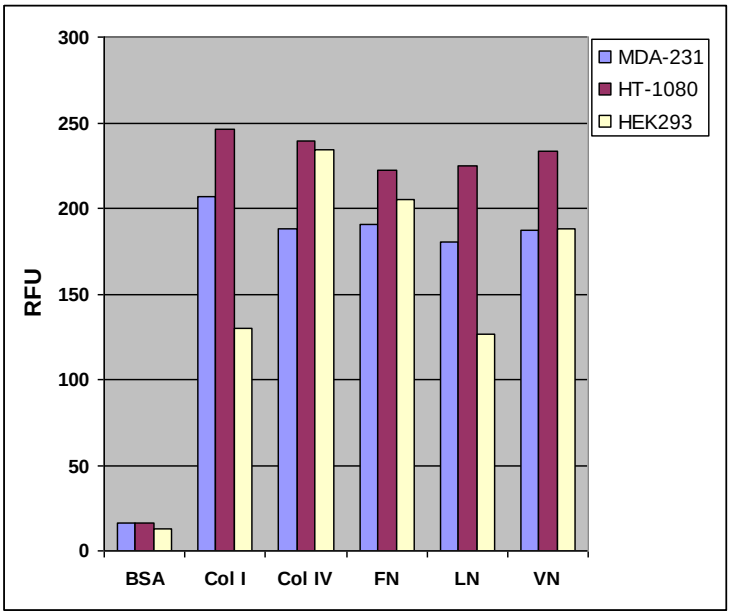

Figure 2. ECM-mediated Cell Adhesion. Serum starved cells were allowed to attach to ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were lysed and quantified by CyQuant® GR Dye as described in the Assay Protocol.

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.

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Recent Product Citations

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5. Maxwell, J.T. et al. (2019). Electrical stimulation of pediatric cardiac-derived c-kit+ progenitor cells improves retention and cardiac function in right ventricular heart failure. *Stem Cells*. doi: 10.1002/stem.3088.
6. Uno, Y. et al. (2019). Increased Expression of DNAJC12 is Associated with Aggressive Phenotype of Gastric Cancer. *Ann Surg Oncol*. **26**(3):836-844. doi: 10.1245/s10434-018-07149-y.
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8. Kim, S.W. et al. (2013). Cardiac stem cells with electrical stimulation improve ischaemic heart function through regulation of connective tissue growth factor and miR-378. *Cardiovasc Res*. 10.1093/cvr/cvt192.

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