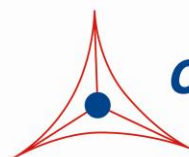

Product Manual

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

Catalog Number

CBA-070	48 assays
CBA-070-5	5 x 48 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
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 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 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-071: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Fluorometric 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. 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 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 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 ECM 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 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 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.

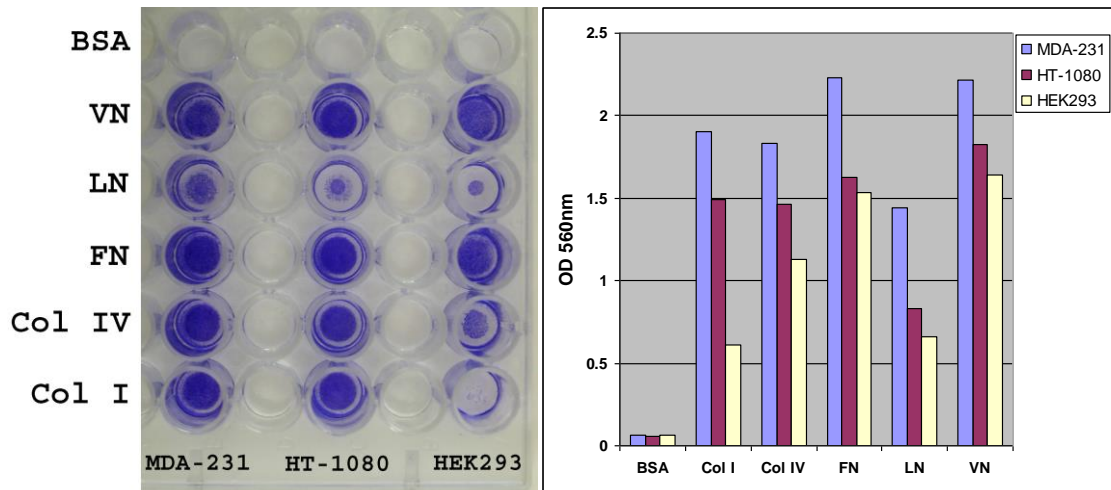


Figure 1. ECM-mediated Cell Adhesion. Serum starved cells were allowed to attach to ECM-coated 48-well plate for 1 hr at 100,000 cells/well. Adherent cells were stained (left panel picture) and quantified at OD 560nm after extraction (right panel figure).

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. Chen, Z. et al. (2023). The alpha7 integrin subunit in astrocytes promotes endothelial blood-brain barrier integrity. *Development*. **150**(6):dev201356. doi: 10.1242/dev.201356.
2. Liu, M.X, et al. (2023). MiR-410-3p suppresses primary gastric cancer and exosomes regulate endogenous expression of miR-410-3p. *Am J Cancer Res.* **13**(6):2670-2680.
3. García-García, T. et al. (2022). Impairment of antiviral immune response and disruption of cellular functions by SARS-CoV-2 ORF7a and ORF7b. *iScience*. doi: 10.1016/j.isci.2022.105444.
4. Khan, A.A. et al. (2022). Determination of anticancer potential of a novel pharmacologically active thiosemicarbazone derivative against colorectal cancer cell lines. *Saudi Pharm J.* doi: 10.1016/j.jsps.2022.03.011.
5. Park, S. et al. (2021). Production of a Foot-and-Mouth Disease Vaccine Antigen Using Suspension-Adapted BHK-21 Cells in a Bioreactor. *Vaccines (Basel)*. **9**(5):505. doi: 10.3390/vaccines9050505.
6. Takeshita, Y. et al. (2021). Angiopoietin-like protein 2 decreases peritoneal metastasis of ovarian cancer cells by suppressing anoikis resistance. *Biochem Biophys Res Commun.* **561**:26-32. doi: 10.1016/j.bbrc.2021.05.008.
7. Potter, J.A. et al. (2021). Collagen I Modifies Connexin-43 Hemichannel Activity via Integrin $\alpha 2\beta 1$ Binding in TGF $\beta 1$ -Evoked Renal Tubular Epithelial Cells. *Int J Mol Sci.* **22**(7):3644. doi: 10.3390/ijms22073644.
8. Liu, S.Y. et al. (2021). Evaluation of Nanotargeted ^{111}In -Cyclic RGDfK-Liposome in a Human Melanoma Xenotransplantation Model. *Int J Mol Sci.* **22**(3):1099. doi: 10.3390/ijms22031099.

9. Iosef, C. et al. (2020). Quantitative proteomics reveal lineage-specific protein profiles in iPSC-derived Marfan syndrome smooth muscle cells. *Sci Rep.* **10**(1):20392. doi: 10.1038/s41598-020-77274-w.
10. Karatsai, O. et al. (2020). Combinatory Treatment of Canavanine and Arginine Deprivation Efficiently Targets Human Glioblastoma Cells via Pleiotropic Mechanisms. *Cells.* **9**(10):E2217. doi: 10.3390/cells9102217.
11. Celentano, A. et al. (2020). Kava constituents exert selective anticancer effects in oral squamous cell carcinoma cells in vitro. *Sci Rep.* **10**(1):15904. doi: 10.1038/s41598-020-73058-4.
12. Jha, S.K. et al. (2020). Enhanced antitumor efficacy of bile acid-lipid complex-anchored docetaxel nanoemulsion via oral metronomic scheduling. *J Control Release.* **328**:368-394. doi: 10.1016/j.jconrel.2020.08.067.
13. Lechertier, T. et al. (2020). Pericyte FAK negatively regulates Gas6/Axl signalling to suppress tumour angiogenesis and tumour growth. *Nat Commun.* **11**(1):2810. doi: 10.1038/s41467-020-16618-6.
14. Bagdonaite, I. et al. (2020). O-glycan initiation directs distinct biological pathways and controls epithelial differentiation. *EMBO Rep.* doi: 10.15252/embr.201948885.
15. Okada, J. et al. (2020). Dapagliflozin Inhibits Cell Adhesion to Collagen I and IV and Increases Ectodomain Proteolytic Cleavage of DDR1 by Increasing ADAM10 Activity. *Molecules.* **25**(3). pii: E495. doi: 10.3390/molecules25030495.
16. Liu, L.Q. et al. (2019). MiR-92a antagonized the facilitation effect of extracellular matrix protein 1 in GC metastasis through targeting its 3'UTR region. *Food Chem Toxicol.* **133**:110779. doi: 10.1016/j.fct.2019.110779.
17. Hou, S. et al. (2019). Integrin $\alpha 5$ promotes migration and cisplatin resistance in esophageal squamous cell carcinoma cells. *Am J Cancer Res.* **9**(12):2774-2788.
18. Uno, Y. et al. (2019). Expression, Function, and Prognostic Value of MAGE-D4 Protein in Esophageal Squamous Cell Carcinoma. *Anticancer Res.* **39**(11):6015-6023. doi: 10.21873/anticancer.13807.
19. Umeda, S. et al. (2019). Fraser extracellular matrix complex subunit 1 promotes liver metastasis of gastric cancer. *Int J Cancer.* doi: 10.1002/ijc.32705.
20. Hirano, K. et al. (2019). LacdiNAcylation of N-glycans in MDA-MB-231 human breast cancer cells results in changes in morphological appearance and adhesive properties of the cells. *Histochem Cell Biol.* doi: 10.1007/s00418-019-01822-3.
21. Ding, L. et al. (2019). Perturbed myoepithelial cell differentiation in BRCA mutation carriers and in ductal carcinoma in situ. *Nat Commun.* **10**(1):4182. doi: 10.1038/s41467-019-12125-5.
22. Birtley, J.R. et al. (2019). Inactivating mutations and X-ray crystal structure of the tumor suppressor OPCML reveal cancer-associated functions. *Nat Commun.* **10**(1):3134. doi: 10.1038/s41467-019-10966-8.
23. Sohn, H.J. et al. (2019). Cellular characterization of actin gene concerned with contact-dependent mechanisms in *Naegleria fowleri*. *Parasite Immunol.* e12631. doi: 10.1111/pim.12631.
24. Babchia, N. et al. (2019). The bidirectional crosstalk between metastatic uveal melanoma cells and hepatic stellate cells engenders an inflammatory microenvironment. *Exp Eye Res.* **181**:213-222. doi: 10.1016/j.exer.2019.02.012.
25. Zhao, X. et al. (2019). Intracellular reduction in ATP levels contributes to CYT997-induced suppression of metastasis of head and neck squamous carcinoma. *J Cell Mol Med.* **23**(2):1174-1182. doi: 10.1111/jcmm.14017.

26. Arzmi, M.H. et al. (2019). Monospecies and polymicrobial biofilms differentially regulate the phenotype of genotype-specific oral cancer cells. *Carcinogenesis*. **40**(1):184-193. doi: 10.1093/carcin/bgy137.
27. Sun, H.F. et al. (2019). Loss of TMEM126A promotes extracellular matrix remodeling, epithelial-to-mesenchymal transition, and breast cancer metastasis by regulating mitochondrial retrograde signaling. *Cancer Lett.* 440-441:189-201. doi: 10.1016/j.canlet.2018.10.018.
28. Itoh, H. et al. (2018). Endometrial stromal cell attachment and matrix homeostasis in abdominal wall endometriomas. *Hum Reprod.* **33**(2):280-291. doi: 10.1093/humrep/dex371.
29. Chong, K. et al. (2021) Inflammation by activated macrophage-like THP-1 cells increases human dura mater cell adhesion with alteration of integrin $\alpha_2 \beta_1$ and matrix metalloproteinase. *J Orthop Res.* **37**(3):706-716. doi: 10.1002/jor.24207.
30. Zhou, M. et al. (2018). Caspase-3 regulates the migration, invasion and metastasis of colon cancer cells. *Int J Cancer.* 143(4):921-930. doi: 10.1002/ijc.31374.

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.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2006-2024: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.