Product Manual

CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric Format)

Catalog Number

CBA-110 **12 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

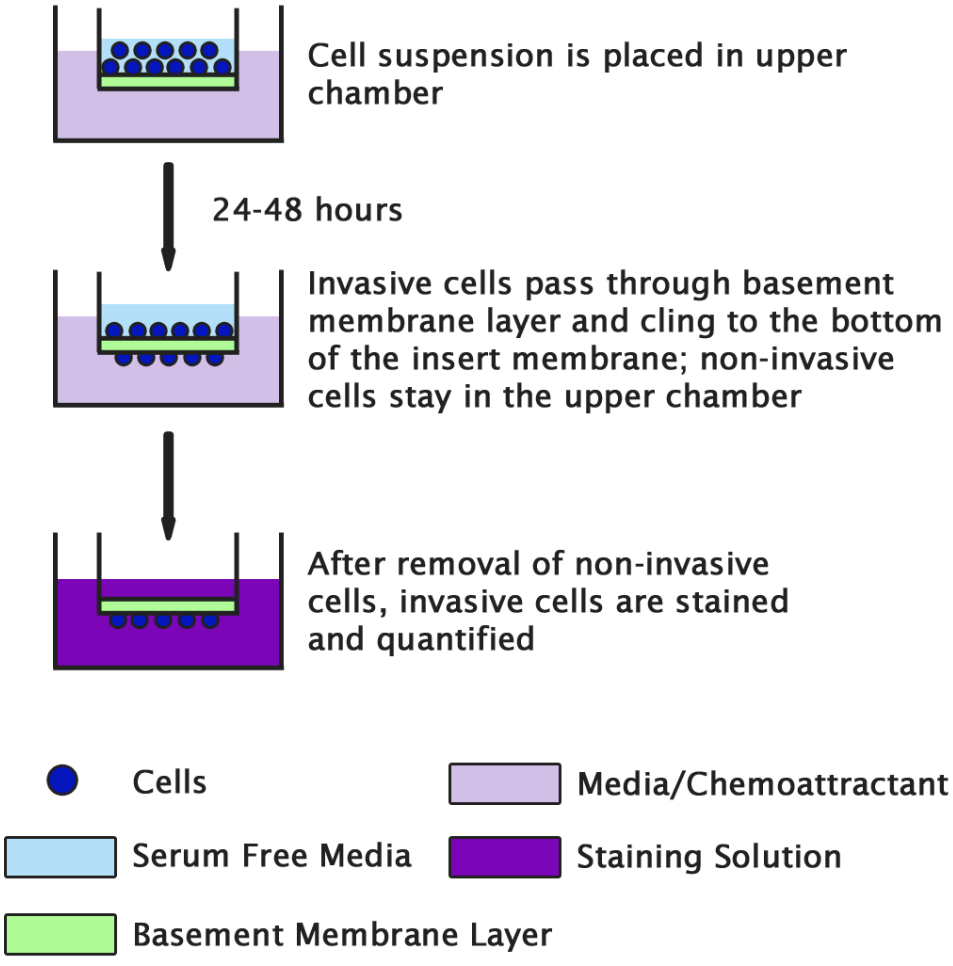
Introduction

The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic cells produce many proteolytic enzymes (e.g. lysosomal hydrolysates, collagenases, plasminogen activators) while the expression of certain cell surface protease receptors is also increased.

Cell Biolabs CytoSelect™ Cell Invasion Assay Kit utilizes basement membrane-coated inserts to assay the invasive properties of tumor cells. It contains sufficient reagents for the evaluation of 12 samples.

Assay Principle

The CytoSelect™ Cell Invasion Assay Kit contains polycarbonate membrane inserts (8 µm pore size) in a 24-well plate. The upper surface of the insert membrane is coated with a uniform layer of dried basement membrane matrix solution. This basement membrane layer serves as a barrier to discriminate invasive cells from non-invasive cells. Invasive cells are able to degrade the matrix proteins in the layer, and ultimately pass through the pores of the polycarbonate membrane. Finally, the cells are removed from the top of the membrane and the invaded cells are stained and quantified.



Related Products

1. CBA-100-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay (8µm, Colorimetric)
2. CBA-110-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Colorimetric)
3. CBA-110-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, Colorimetric)
4. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
5. CBA-112-COL: CytoSelect™ 96-Well Cell Invasion Assay (Collagen I, Fluorometric)

Kit Components

1. ECM Invasion Chamber Plate (Part No. 11001): One 24-well plate containing 12 ECM-coated cell culture inserts.
2. Cell Stain Solution (Part No. 11002): One 10 mL bottle
3. Extraction Solution (Part No. 11003): One 10 mL bottle
4. Cotton Swabs: (Part No. 11004) 40 each
5. Forceps: (Part No. 11005) One each

Materials Not Supplied

1. Invasive cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Microtiter plate reader

Storage

Store all components at 4ºC.

Assay Protocol

1. Under sterile conditions, allow the invasion chamber plate to warm up at room temperature for 10 minutes.
2. Rehydrate the basement membrane layer of the cell culture inserts by adding 300 µL of warm, serum-free media to the inner compartment. Incubate at room temperature for 1 hour.
3. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/ml in serum free media. Agents that inhibit or stimulate cell invasion can be added directly to the cell suspension.
   Note: Overnight starvation may be performed prior to running the assay

4. Carefully remove the rehydration medium (step 2) from the inserts without disturbing the basement membrane layer.
   Note: It will not affect the assay performance if a small amount of rehydration medium is left in the compartment
5. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
6. Add 300 µL of the cell suspension solution to the inside of each insert.
7. Incubate for 24-48 hours at 37ºC in 5% CO_2 atmosphere.
8. Carefully aspirate the media from the inside of the insert. Wet the ends of 2-3 cotton-tipped swabs with water, flatten the ends of the swabs by pressing them against a clean hard surface, and gently swab the interior of the inserts to remove non-invasive cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.
9. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
10. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
11. (optional) Count invasive cells with a light microscope under high magnification objective, with at least three individual fields per insert.
12. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
13. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

Example of Results

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

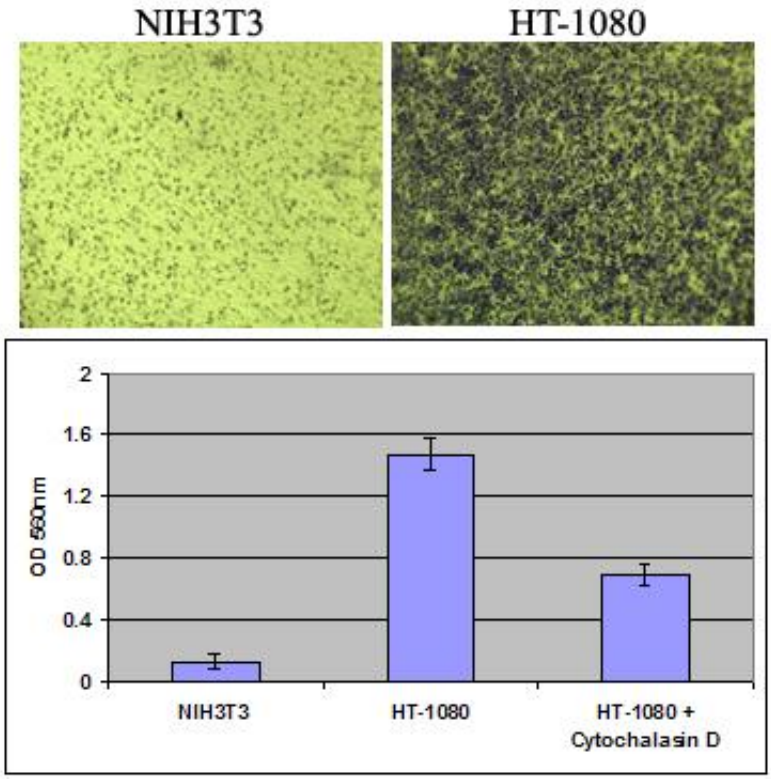


Figure 1. Human Fibrosarcoma HT-1080 Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 300,000 cells/well and allowed to invade toward 10% FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture) and quantified at OD 560nm after extraction (bottom panel figure).

References

1. Erkell, L. J., Schirrmacher, V. (1988) *Cancer Res* **48**, 6933-6937.
2. Montgomery, A. M. P., De Clerck, Y. A., Langley, K. E., Reisfeld, R. A., Mueller, B. M. (1993) *Cancer Res* **53**,693-700.
3. Monsky, W. L., Lin, C. Y., Aoyama, A., Kelly, T., Akiyama, S. K., Mueller, S. C., Chen, W. T. (1994) *Cancer Res* **54**,5702-5710.

Recent Product Citations

1. Kahm, Y.J. et al. (2023). RanBP1: A Potential Therapeutic Target for Cancer Stem Cells in Lung Cancer and Glioma. *Int J Mol Sci*. **24**(7):6855. doi: 10.3390/ijms24076855.
2. Houri, A. et al. (2023). Suprabasin enhances the invasion, migration, and angiogenic ability of oral squamous cell carcinoma cells under hypoxic conditions. *Oncol Rep*. **49**(5):83. doi: 10.3892/or.2023.8520.
3. Fan, Y. et al. (2023). hPSC-derived sacral neural crest enables rescue in a severe model of Hirschsprung's disease. *Cell Stem Cell*. **30**(3):264-282.e9. doi: 10.1016/j.stem.2023.02.003.
4. Garcia, J.A. et al. (2023). Acss2/HIF-2 signaling facilitates colon cancer growth and metastasis. *PLoS One*. **18**(3):e0282223. doi: 10.1371/journal.pone.0282223.

5. Nunomura, J. et al. (2022). Interleukin-1β triggers matrix metalloprotease-3 expression through p65/RelA activation in melanoma cells. *PLoS One*. **17**(11):e0278220. doi: 10.1371/journal.pone.0278220.
6. Mucha, B. et al. (2022). Tumor suppressor mediated ubiquitylation of hnRNPK is a barrier to oncogenic translation. *Nat Commun*. **13**(1):6614. doi: 10.1038/s41467-022-34402-6.
7. Polo-Generelo, S. et al. (2022). TGF-β-Upregulated Lnc-Nr6a1 Acts as a Reservoir of miR-181 and Mediates Assembly of a Glycolytic Complex. *Noncoding RNA*. **8**(5):62. doi: 10.3390/ncrna8050062.
8. Hino, S.I. et al. (2022). Suppression of HCT116 Human Colon Cancer Cell Motility by Polymethoxyflavones is Associated with Inhibition of Wnt/β-Catenin Signaling. *Nutr Cancer*. doi: 10.1080/01635581.2022.2084122.
9. Feliz Morel, Á.J. et al. (2022). Persistent Properties of a Subpopulation of Cancer Cells Overexpressing the Hedgehog Receptor Patched. *Pharmaceutics*. **14**(5):988. doi: 10.3390/pharmaceutics14050988.
10. Ishihara, S. et al. (2022). The lactate sensor GPR81 regulates glycolysis and tumor growth of breast cancer. *Sci Rep*. **12**(1):6261. doi: 10.1038/s41598-022-10143-w.
11. Haimovici, A. et al. (2022). Spontaneous activity of the mitochondrial apoptosis pathway drives chromosomal defects, the appearance of micronuclei and cancer metastasis through the Caspase-Activated DNAse. *Cell Death Dis*. **13**(4):315. doi: 10.1038/s41419-022-04768-y.
12. Sato, N. et al. (2022). Tumor-suppressive role of Smad ubiquitination regulatory factor 2 in patients with colorectal cancer. *Sci Rep*. **12**(1):5495. doi: 10.1038/s41598-022-09390-8.
13. Pospiech, K. et al. (2022). TGFα-EGFR pathway in breast carcinogenesis, association with WWOX expression and estrogen activation. *J Appl Genet*. **63**(2):339-359. doi: 10.1007/s13353-022-00690-3.
14. Sato, N. et al. (2022). Yin Yang 1 regulates ITGAV and ITGB1, contributing to improved prognosis of colorectal cancer. *Oncol Rep*. **47**(5):87. doi: 10.3892/or.2022.8298.
15. Park, J.S. et al. (2022). Gene Expression Analysis of Aggressive Adult Xp11.2 Translocation Renal Cell Carcinoma at Clinical Stage T1N0M0 to Identify Potential Prognostic and Therapeutic Biomarkers. *Biomedicines*. **10**(2):321. doi: 10.3390/biomedicines10020321.
16. Tai, Y.K. et al. (2022). Modulated TRPC1 Expression Predicts Sensitivity of Breast Cancer to Doxorubicin and Magnetic Field Therapy: Segue Towards a Precision Medicine Approach. *Front Oncol*. **11**:783803. doi: 10.3389/fonc.2021.783803.
17. Leggett, C.S. et al. (2021). Identification and characterization of potent, selective, and efficacious inhibitors of human arylamine N-acetyltransferase 1. *Arch Toxicol*. doi: 10.1007/s00204-021-03194-x.
18. Zhang, J. et al. (2021). Wnt2 Contributes to the Development of Atherosclerosis. *Front Cardiovasc Med*. **8**:751720. doi: 10.3389/fcvm.2021.751720.
19. Zhang, N. et al. (2021). LncRNA FGD5-AS1 functions as an oncogene to upregulate GTPBP4 expression by sponging miR-873-5p in hepatocellular carcinoma. *Eur J Histochem*. **65**(4). doi: 10.4081/ejh.2021.3300.
20. Martínez-López, A. et al. (2021). Inhibition of RAC1 activity in cancer associated fibroblasts favours breast tumour development through IL-1β upregulation. *Cancer Lett*. **521**:14-28. doi: 10.1016/j.canlet.2021.08.014.
21. Avşar Abdik, E. (2021). Differentiated pre-adipocytes promote proliferation, migration and epithelial-mesenchymal transition in breast cancer cells of different p53 status. *Mol Biol Rep*. doi: 10.1007/s11033-021-06521-8.

22. Chen, H. et al. (2021). Signaling of MK2 sustains robust AP1 activity for triple negative breast cancer tumorigenesis through direct phosphorylation of JAB1. *NPJ Breast Cancer*. **7**(1):91. doi: 10.1038/s41523-021-00300-1.
23. Huang, J. et al. (2021). Long non-coding RNA 00858 knockdown alleviates bladder cancer via regulation of the miR-3064-5p/CTGF axis. *Oncol Rep*. **46**(2):164. doi: 10.3892/or.2021.8115.
24. Alburquerque-González, B. et al. (2021). The FDA-Approved Antiviral Raltegravir Inhibits Fascin1-Dependent Invasion of Colorectal Tumor Cells In Vitro and In Vivo. *Cancers*. **13**(4):861. doi: 10.3390/cancers13040861.
25. Shimizu, K. et al. (2020). ARHGAP29 expression may be a novel prognostic factor of cell proliferation and invasion in prostate cancer. *Oncol Rep*. doi: 10.3892/or.2020.7811.
26. Joseph, C. et al. (2020). The ITIM-Containing Receptor: Leukocyte-Associated Immunoglobulin-Like Receptor-1 (LAIR-1) Modulates Immune Response and Confers Poor Prognosis in Invasive Breast Carcinoma. *Cancers (Basel)*. **13**(1):E80. doi: 10.3390/cancers13010080.
27. Bastos, D.C. et al. (2020). Genetic ablation of FASN attenuates the invasive potential of prostate cancer driven by Pten loss. *J Pathol*. doi: 10.1002/path.5587.
28. Lim, W.C. et al. (2020). Polysaccharide isolated from persimmon leaves (Diospyros kaki Thunb.) suppresses TGF-β1-induced epithelial-to-mesenchymal transition in A549 cells. *Int J Biol Macromol*. S0141-8130(20)34246-X. doi: 10.1016/j.ijbiomac.2020.08.155.
29. Daso, R.E. et al. (2020). Self-Assembled Peptide Based Biocomposites for Near Infra-red Light Triggered Drug Release to Tumor Cells. *Biotechnol J*. doi: 10.1002/biot.202000128.
30. Tang, H. et al (2020). MiR-4328 inhibits proliferation, metastasis and induces apoptosis in keloid fibroblasts by targeting BCL2 expression. *Open Life Sci*. **15**(1):638-646. doi: 10.1515/biol-2020-0056.

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