Product Manual

CytoSelect™ 96-Well Cell Invasion Assay (Collagen I, Fluorometric Format)

Catalog Number

CBA-112-COL **96 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

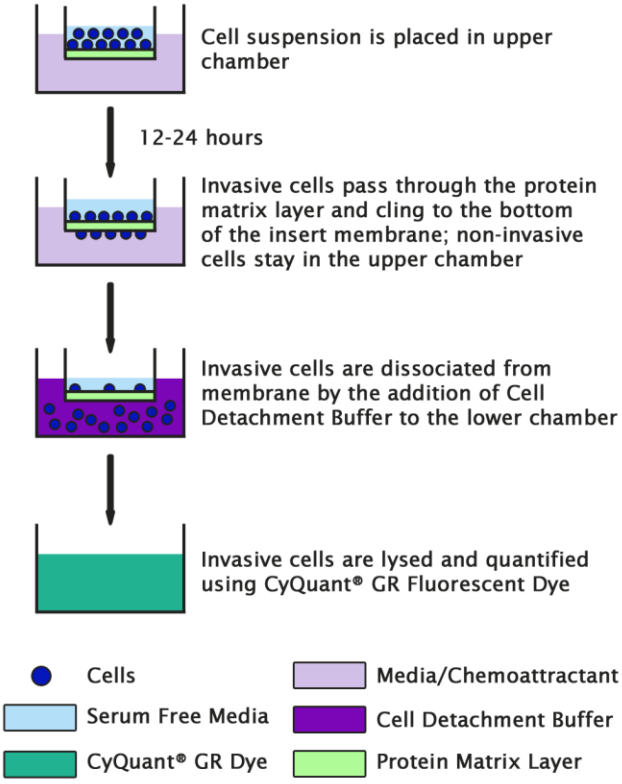
The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic 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™ 96-well Collagen Cell Invasion Assay Kit utilizes Bovine Type I Collagen-coated inserts to assay the invasive properties of tumor cells. The kit does not require you to prelabel the cells with Calcein AM or remove non-invaded cells (i.e. cotton swabbing). Any invaded cells are first dissociated from the membrane, then lysed and detected with CyQuant® GR Dye.

The CytoSelect™ 96-well Collagen Cell Invasion Assay Kit provides a robust system for the quantitative determination of cell invasion. It contains sufficient reagents for the evaluation of 96 samples.

Assay Principle

The CytoSelect™ 96-well Collagen Cell Invasion Assay Kit contains polycarbonate membrane inserts (8 µm pore size) in a 96-well plate. The upper surface of the insert membrane is coated with a uniform layer of dried Bovine Type I Collagen matrix. This collagen matrix layer serves as a barrier to discriminate invasive cells from non-invasive cells. Invasive cells are able to degrade the collagen matrix layer, and ultimately pass through the pores of the polycarbonate membrane. Finally, these invaded cells are then dissociated from the membrane and subsequently detected by the patented CyQuant® GR Dye (Invitrogen).



Related Products

1. CBA-101-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
2. CBA-106-C: CytoSelect™ 96- Well Cell Migration and Invasion Assay (8µm, Fluorometric)
3. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
4. CBA-111-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Fluorometric)
5. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)

Kit Components (shipped at room temperature)

1. 96-well Collagen Invasion Plate (Part No. 111201-COL): One sterile 96-well plate containing collagen-coated inserts (see Figure 1 for components)
2. 96-well Cell Harvesting Tray (Part No. 10402): One 96-well tray
3. Cell Detachment Solution (Part No. 10403): One 20 mL bottle
4. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
5. CyQuant® GR Dye (Part No. 10105): One 75 µL tube

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

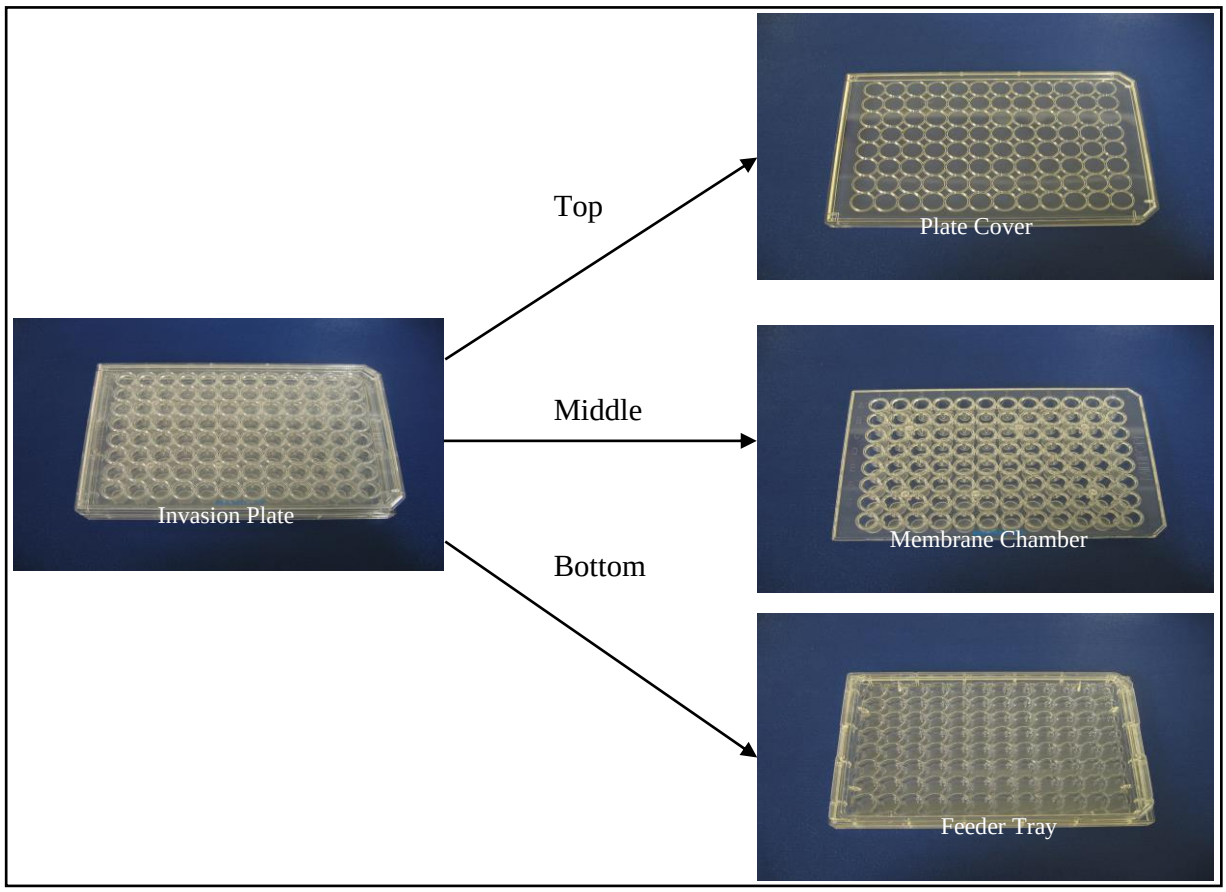


Figure 1: **Components of the 96-well Collagen Cell Invasion Plate.**

Storage

Store all components at 4ºC.

Assay Protocol

1. Under sterile conditions, allow the collagen invasion plate to warm up at room temperature for 10 minutes.
2. Rehydrate the collagen layer of the membrane inserts by adding 125 µL of warm, serum-free media to the inner compartment. Incubate at room temperature for 30 minutes.
3. Prepare a cell suspension containing 0.2-2.0 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 100 µL of the rehydration medium (step 2) from the inserts without disturbing the collagen layer (leaving 25 µL inside).
5. Under sterile conditions, separate the cover and membrane chamber from the feeder tray. Add 150 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the wells of the feeder tray.
6. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). **Ensure no bubbles are trapped under the membrane.**

7. Gently mix the cell suspension from step 3 and add 100 µL to the membrane chamber.
8. Finally, cover the plate and transfer to a cell culture incubator for 12-24 hours.
9. Just prior to the end of the incubation, pipette 150 µL of prewarmed Cell Detachment Solution into wells of the clean, 96-Well Cell Harvesting Tray (provided).
10. Carefully remove the 96-well Invasion Plate from the incubator. Separate the membrane chamber from the feeder tray.
11. Remove the cells/media from the top side of the membrane chamber by aspirating or inverting. Place the membrane chamber into the Cell Harvesting Tray containing 150 µL of Cell Detachment Solution (step 9). Incubate 30 minutes at 37ºC.
12. Completely dislodge the cells from the underside of the membrane by gently tilting the membrane chamber several times in the Cell Detachment Solution.
13. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:75 in 4X Lysis Buffer (for example, add 5 µL dye to 370 µL of 4X Lysis Buffer).
14. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of Cell Detachment Solution). Incubate 20 minutes at room temperature.
15. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorescence plate reader at 480 nm/520 nm.

Example of Results

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

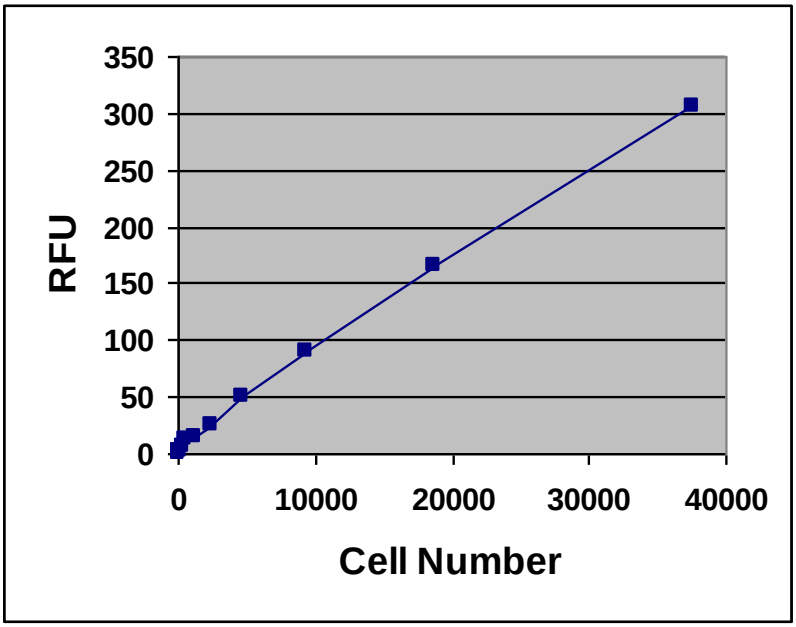


Figure 2: **Quantitation of Human HT-1080**. HT-1080 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150µL cell suspension was mixed with 50µL of 4X Lysis Buffer/dye).

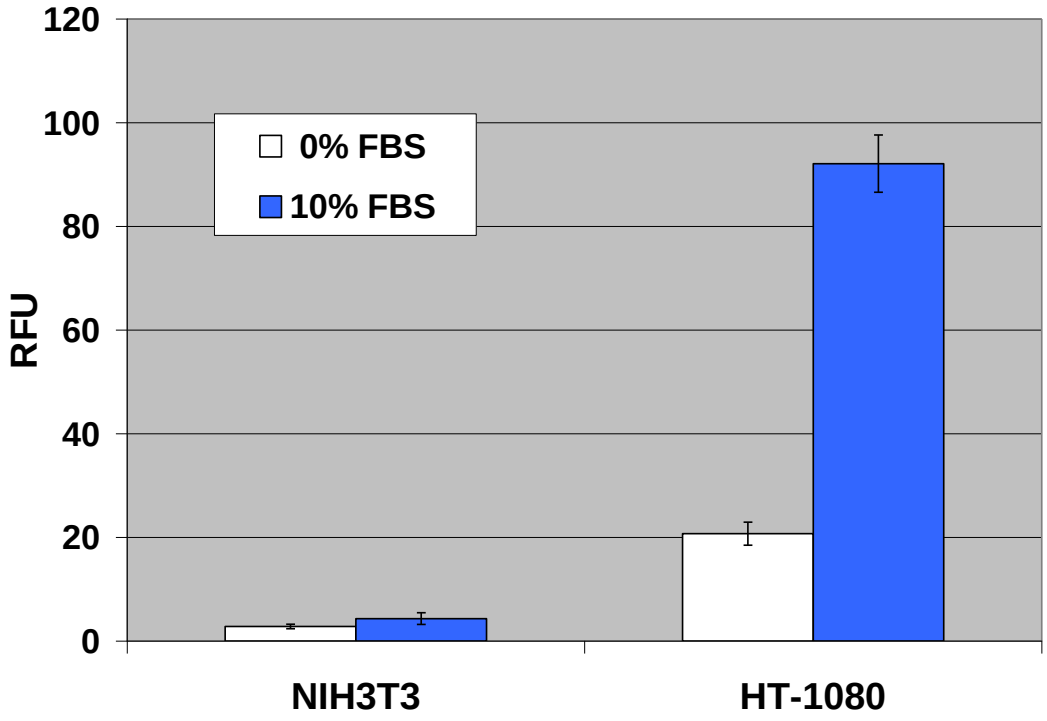


Figure 3: Human Fibrosarcoma HT-1080 Collagen Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 70,000 cells/well and allowed to invade toward FBS for 24 hrs. Invasive cells on the bottom of the invasion membrane were quantified by CyQuant® GR dye as described in the Assay Protocol.

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. Negro, G. et al. (2020). Molecular heterogeneity in breast carcinoma cells with increased invasive capacities. *Radiol Oncol*. **54**(1):103-118. doi: 10.2478/raon-2020-0007.
2. Matsui, A. et al. (2018). Prolonged engraftment of transplanted hepatocytes in the liver by transient pro-survival factor supplementation using ex vivo mRNA transfection. *J Control Release*. **285**:1-11. doi: 10.1016/j.jconrel.2018.06.033.
3. Ohishi, T. et al. (2017). Tankyrase-binding protein TNKS1BP1 regulates actin cytoskeleton rearrangement and cancer cell invasion. *Cancer Res*. 1846.2016. doi: 10.1158/0008-5472.CAN-16-1846.
4. Ben-David, U. et al. (2016). The landscape of chromosomal aberrations in breast cancer mouse models reveals driver-specific routes to tumorigenesis. *Nat Commun*. doi:10.1038/ncomms12160.

5. Ho, P. W. et al. (2015). Knockdown of PTHR1 in osteosarcoma cells decreases invasion and growth and increases tumor differentiation in vivo. *Oncogene.* **34**:2922-2933.
6. Ishiba, T. e. al. (2014). Periostin suppression induces decorin secretion leading to reduced breast cancer cell motility and invasion. *Sci Rep*. **4**:7069.
7. Ziemann, A. et al. (2013). CRN2 enhances the invasiveness of glioblastoma cells. *Neuro Oncology* 10.1093/neuonc/nos388.
8. Liu, X. et al. (2013). Antiproliferative, antiinvasive, and proapoptotic activity of folate receptor α-targeted liposomal doxorubicin in nonfunctional pituitary adenoma cells. *Endocrinology* **154**:1414-1423.
9. Ziemann, A. et al. (2013). CRN2 enhances the invasiveness of glioblastoma cells. *Neuro Oncology* 10.1093/neuonc/nos388.

License Information

This product is provided under an intellectual property license from Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment; (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad CA 92008 USA or outlicensing@lifetech.com.

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