
Product Manual

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

Catalog Number

CBA-112-LN

96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures



CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

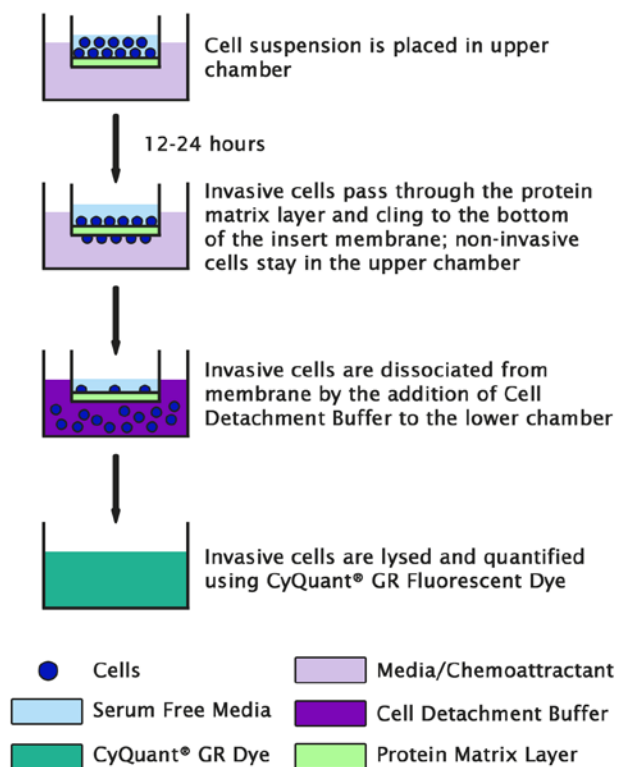
The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic 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 Laminin Cell Invasion Assay Kit utilizes Murine Laminin I-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.

Cell Biolabs CytoSelect™ 96-well Laminin 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 Laminin 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 Murine Laminin I matrix. This laminin matrix layer serves as a barrier to discriminate invasive cells from non-invasive cells. Invasive cells are able to degrade the laminin 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-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
2. CBA-106-C: CytoSelect™ 96-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
3. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
4. CBA-110-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Colorimetric)
5. CBA-110-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, Colorimetric)
6. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
7. CBA-111-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Fluorometric)
8. CBA-111-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, Fluorometric)
9. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
10. CBA-112-COL: CytoSelect™ 96-Well Cell Invasion Assay (Collagen I, Fluorometric)
11. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

1. 96-well Laminin Invasion Plate (Part No. 111201-LN): One sterile 96-well plate containing laminin-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 CaCl₂ and 2 mM MgCl₂
4. Cell culture incubator (37°C, 5% CO₂ atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Microtiter plate reader

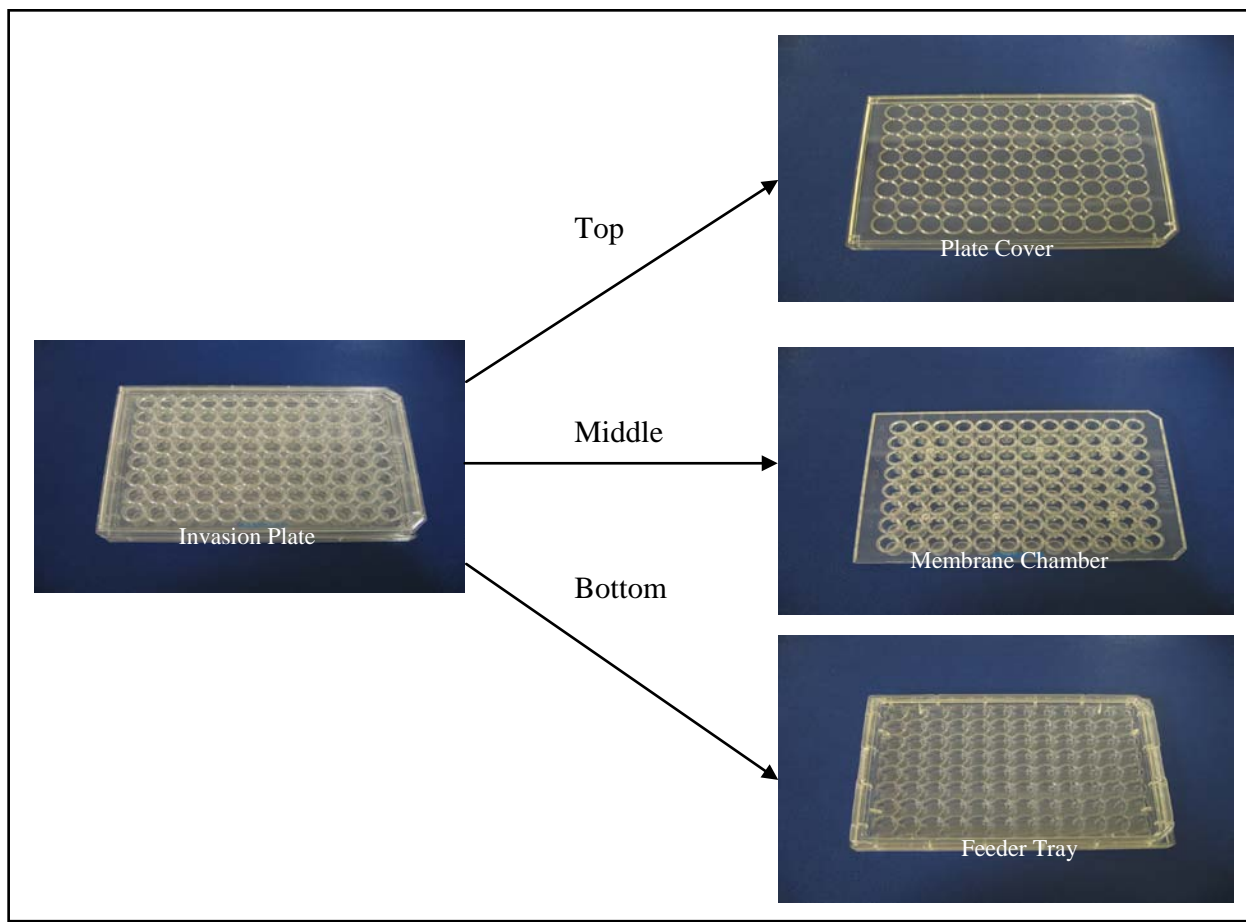


Figure 1: Components of the 96-well Collagen Cell Invasion Plate.

Storage

Store all components at 4°C.

Assay Protocol

1. Under sterile conditions, allow the laminin invasion plate to warm up at room temperature for 10 minutes.
2. Rehydrate the laminin 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 $0.2\text{-}2.0 \times 10^6$ 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 laminin 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 37°C.
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. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

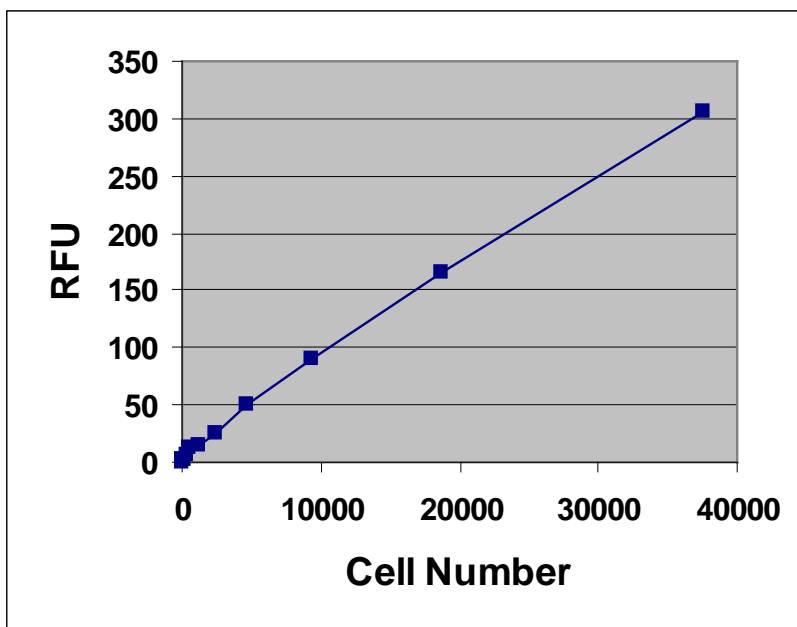


Figure 2: Quantitation of Human HT-1080. HT-1080 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150µL cell suspension was mixed with 50µL of 4X Lysis Buffer/dye).

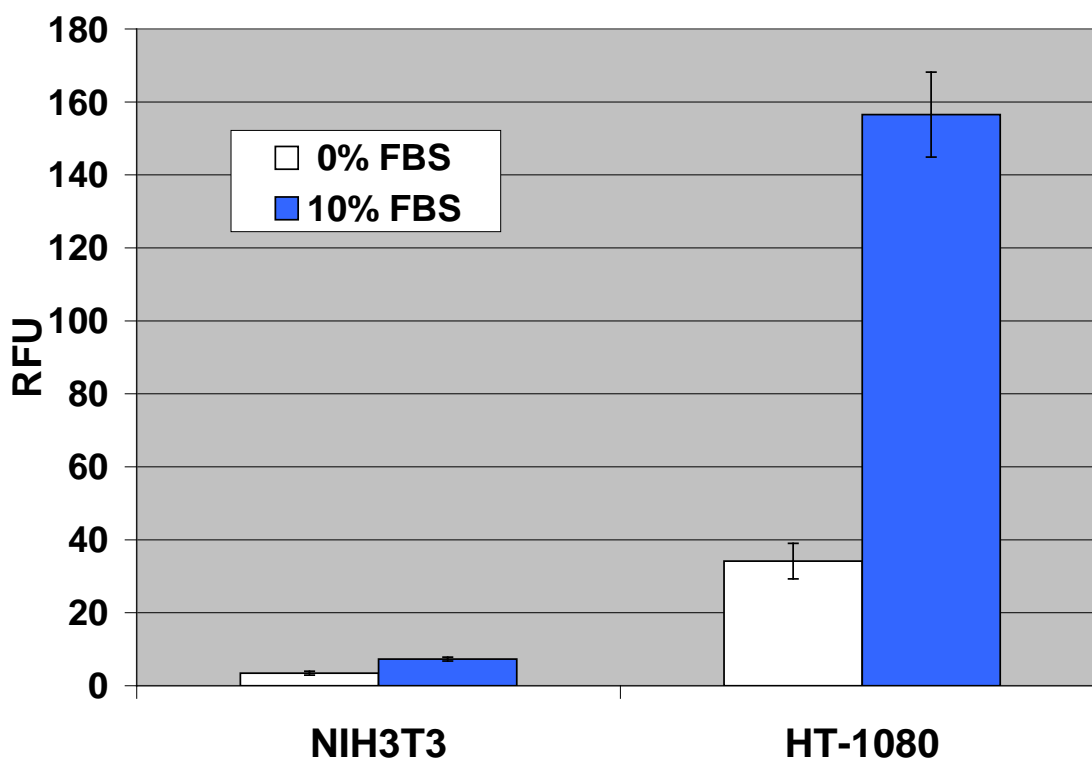


Figure 3: Human Fibrosarcoma HT-1080 Laminin Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 100,000 cells/well and allowed to invade toward FBS for 24 hrs. Invasive cells on the bottom of the invasion membrane were quantified by CyQuant® GR dye as described in the Assay Protocol.

References

1. Erkell, L. J., Schirmacher, 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.

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.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2006-2016: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.