Product Manual

CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric Format)

Catalog Number

CBA-112	96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

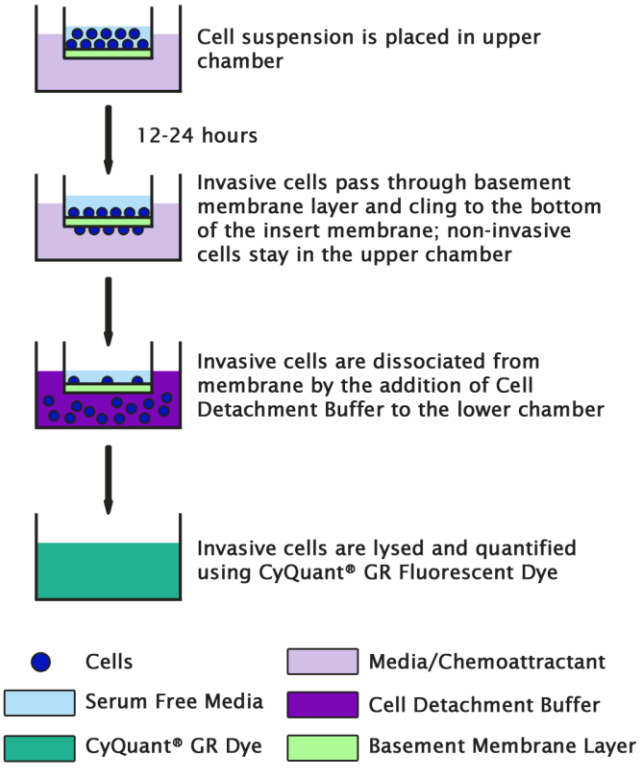
The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic 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 Cell Invasion Assay Kit utilizes basement membrane-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 Cell Invasion Assay Kit provides a robust system for the quantitative determination of cell invasion. The kit contains sufficient reagents for the evaluation of 96 samples.

Assay Principle

The CytoSelect™ 96-well 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 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 invaded cells are dissociated from the membrane and subsequently detected with 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-COL: CytoSelect™ 96-Well Cell Invasion Assay (Collagen I, Fluorometric)

Kit Components

1. 96-well ECM Invasion Plate (Part No. 11201): One sterile 96-well plate containing ECM-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

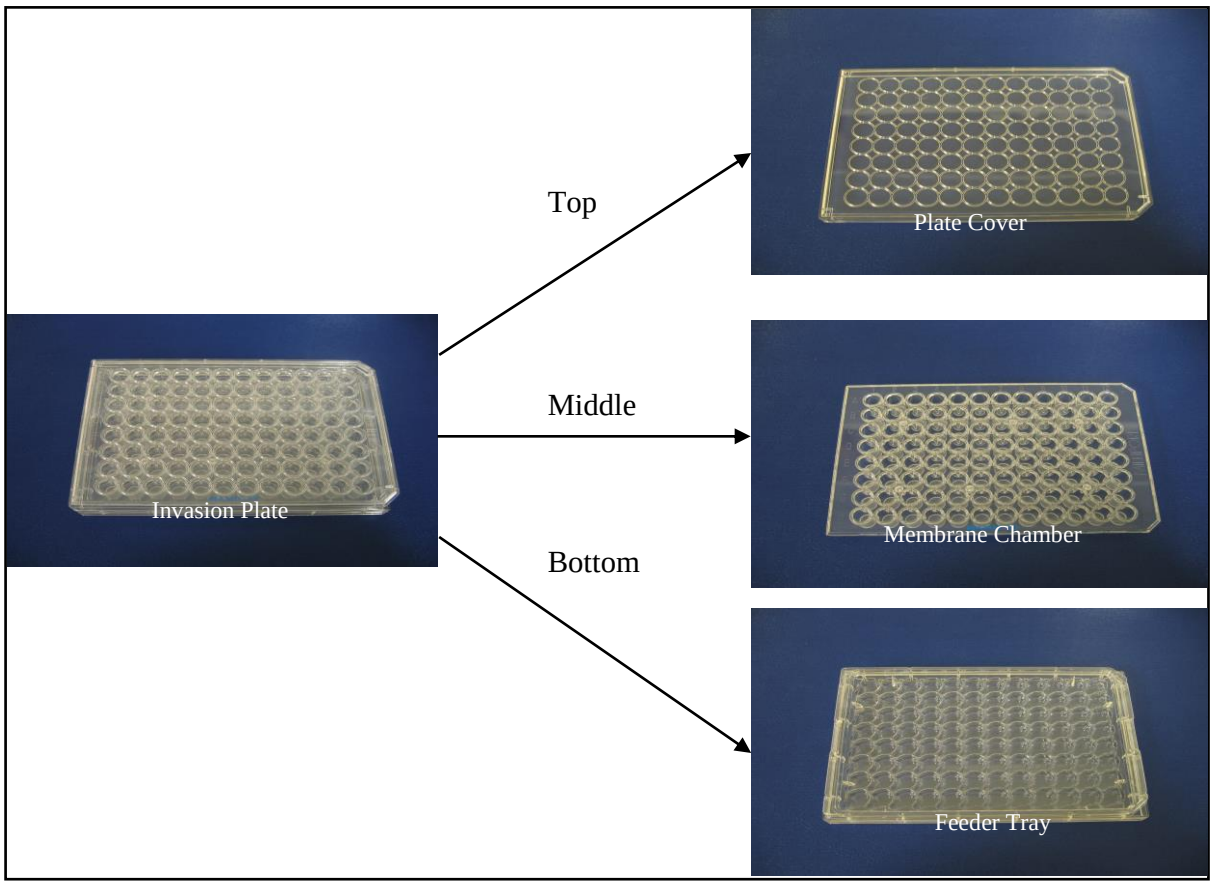


Figure 1: **Components of the 96-well ECM Invasion Plate.**

Storage

Store all components at 4ºC.

Assay Protocol

1. Under sterile conditions, allow the invasion plate to warm up at room temperature for 10 minutes.
2. Rehydrate the basement membrane layer of the membrane inserts by adding 100 µL of warm, serum-free media to the inner compartment. Incubate at room temperature for 1 hour.
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.
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. 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™ 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.

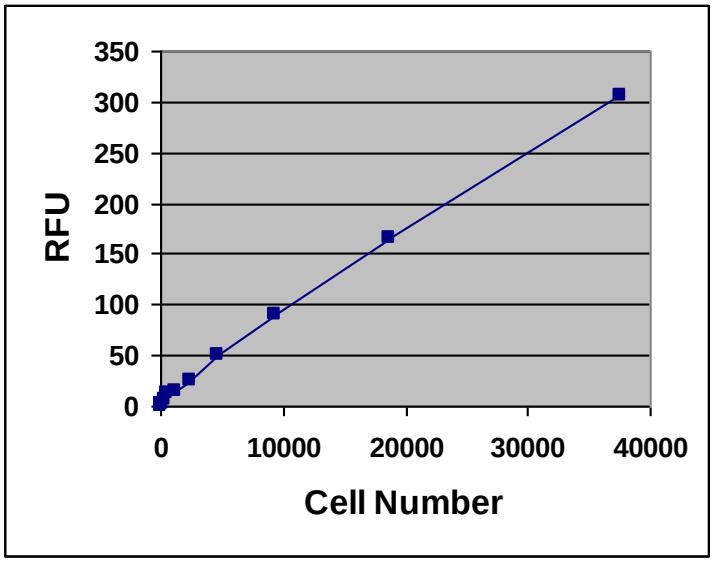


Figure 2: **Quantitation of Human HT-1080**. HT-1080 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150µL cell suspension was mixed with 50µL of 4X Lysis Buffer/dye).

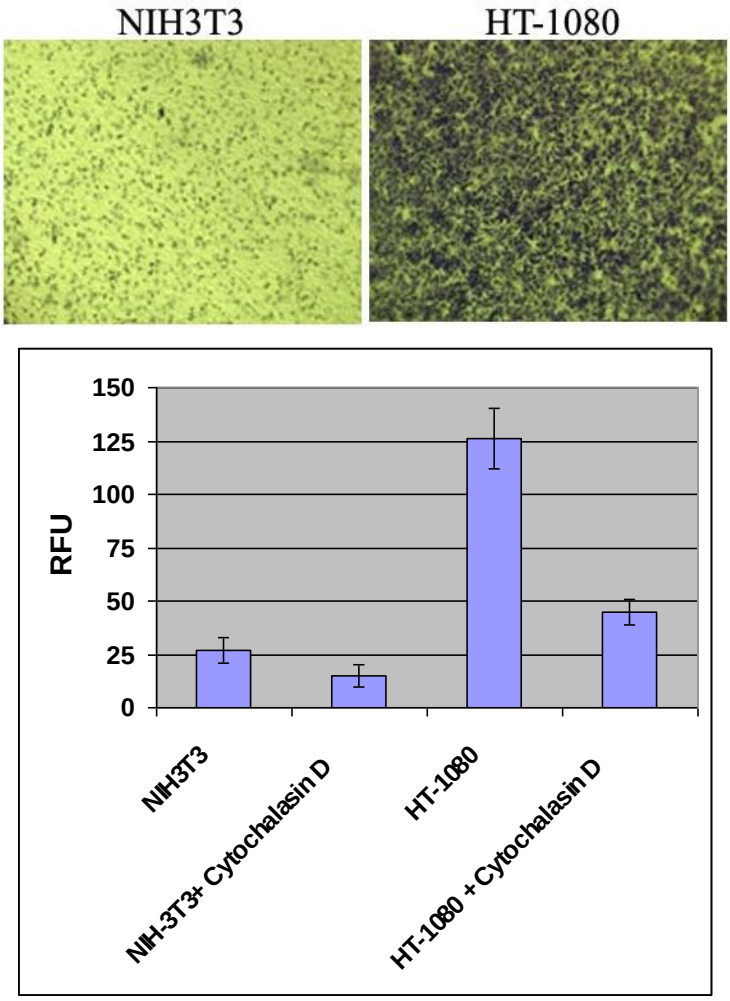


Figure 3: HT-1080 Invasion Assay. HT-1080 or NIH3T3 cells were allowed to invade toward 10% FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D, 200,000 cells were used in each assay. Invaded cells on the bottom of the polycarbonate membrane were stained (top) and quantified by CyQuant® GR Dye as described in Assay Protocol.

References

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Recent Product Citations

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Warranty

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