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Product Manual

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

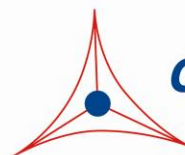
Catalog Number

CBA-111

12 assays

**FOR RESEARCH USE ONLY**  
**Not for use in diagnostic procedures**

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*Creating Solutions for Life Science Research*

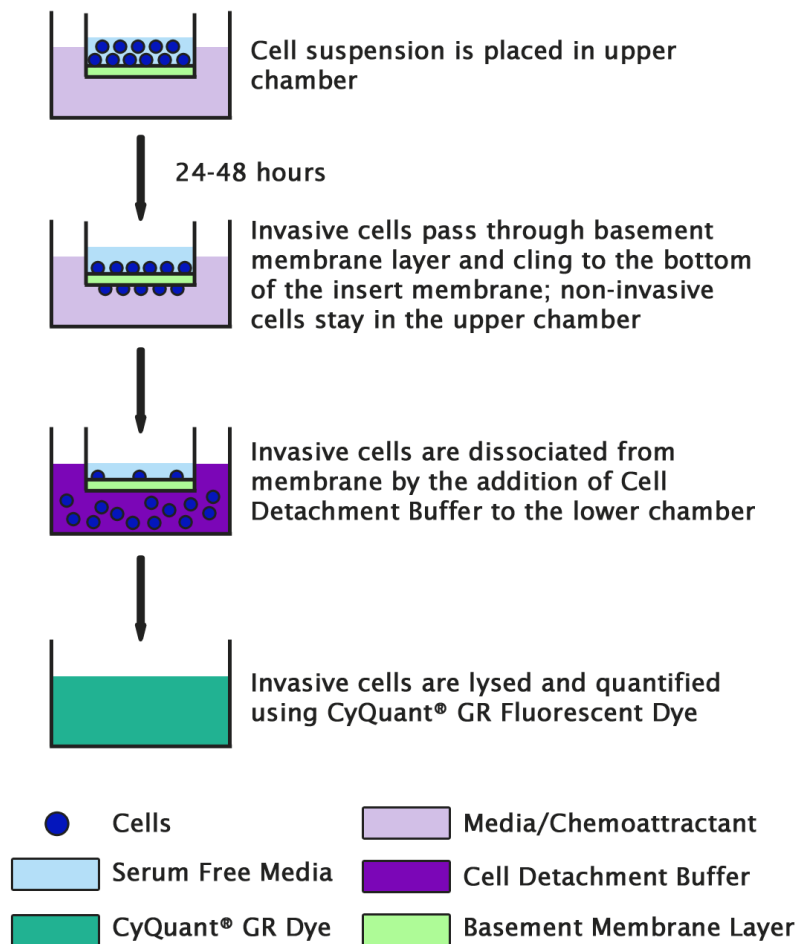
## **Introduction**

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, these cells are 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-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Fluorometric)
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 Detachment Solution (Part No. 10101): One 5 mL bottle
3. 4X Lysis Buffer (Part No. 10102): One 5 mL bottle
4. CyQuant® GR Dye: (Part No. 10103) One 25 µL tube
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<sub>2</sub> and 2 mM MgCl<sub>2</sub>
4. Cell culture incubator (37°C, 5% CO<sub>2</sub> atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Fluorescence plate reader

## **Storage**

Store all components at 4°C.

## **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<sup>6</sup> 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*

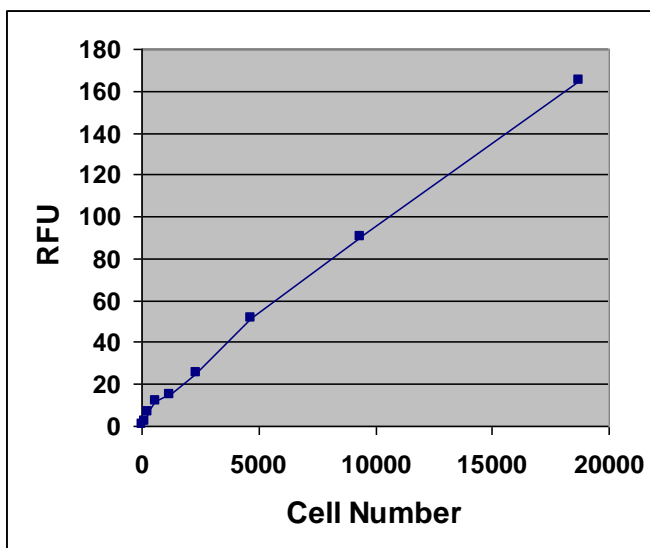
- Carefully remove the rehydration medium (step 2) from the inserts without disturbing the basement membrane layer.

*Note: It will not affect the assay performance if a small amount of rehydration medium is left in the compartment*

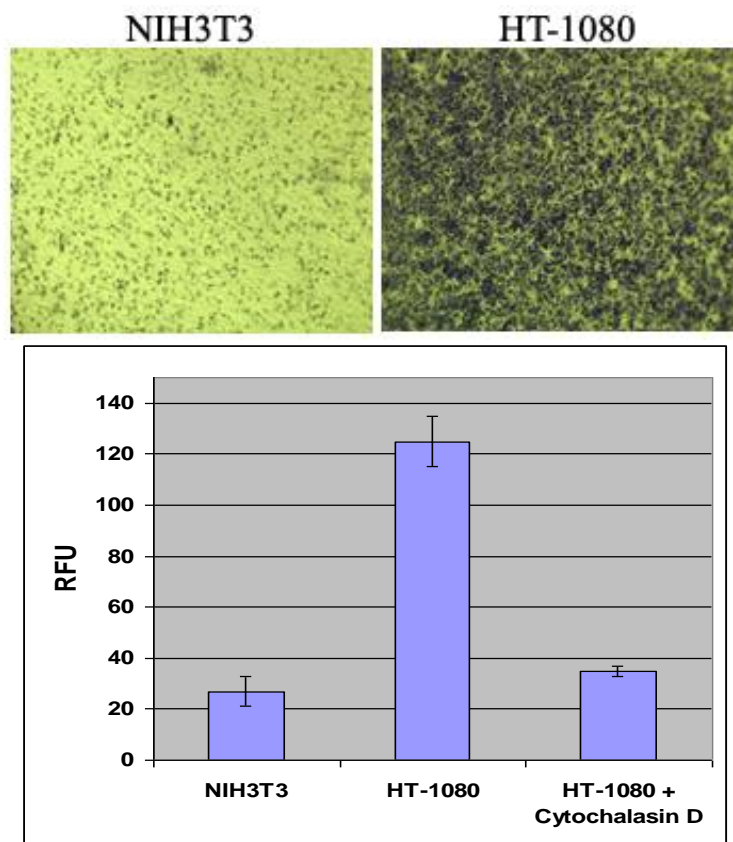
- Add 500  $\mu\text{L}$  of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
- Add 300  $\mu\text{L}$  of the cell suspension solution to the inside of each insert.
- Incubate for 24-48 hours at 37°C in 5%  $\text{CO}_2$  atmosphere.
- Carefully aspirate the media from the inside of the insert. Transfer the insert to a clean well containing 225  $\mu\text{L}$  of Cell Detachment Solution. Incubate 30 minutes at 37°C.
- Completely dislodge the cells from the underside of the membrane by gently tilting the insert several times in the detachment solution. Remove and discard the insert.
- 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  $\mu\text{L}$  dye to 370  $\mu\text{L}$  of 4X Lysis Buffer).
- Add 75  $\mu\text{L}$  of 4X Lysis Buffer/CyQuant® GR dye solution to each well containing cells and 225  $\mu\text{L}$  of Cell Detachment Solution. Incubate 20 minutes at room temperature.
- Transfer 200  $\mu\text{L}$  of the mixture to a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480nm/520nm.

### **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: Quantitation of Human HT-1080.** HT-1080 cell suspension was titrated in Cell Detachment Buffer; 150 $\mu\text{L}$  of the diluted cell suspension was mixed with 50 $\mu\text{L}$  of 4X Lysis Buffer/CyQuant® GR Dye (1:75).



**Figure 2. 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  $\mu$ M Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture) and quantified by CyQuant® GR Dye (bottom panel figure).

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