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Product Manual

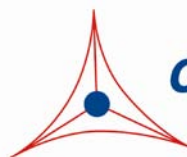
# CytoSelect™ 96- Well Cell Invasion Assay (Collagen I, Fluorometric Format)

Catalog Number

CBA- 112- COL      96 assays

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

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**CELL BIOLABS, INC.**

*Creating Solutions for Life Science Research*

## **Introduction**

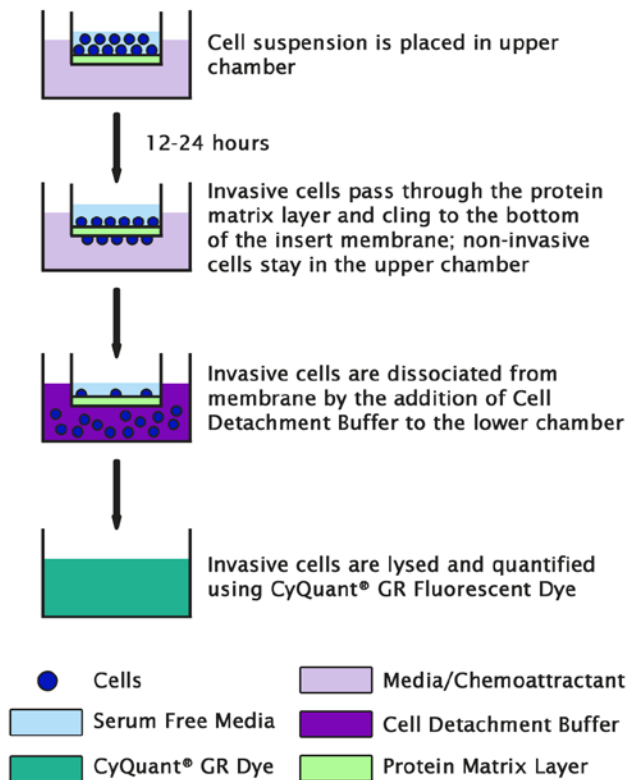
The ability of malignant tumor cells to invade normal surrounding tissue contributes in large part to the significant morbidity and mortality of cancers. Invasiveness requires several distinct cellular functions including adhesion, motility, detachment, and extracellular matrix proteolysis. Metastatic 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**

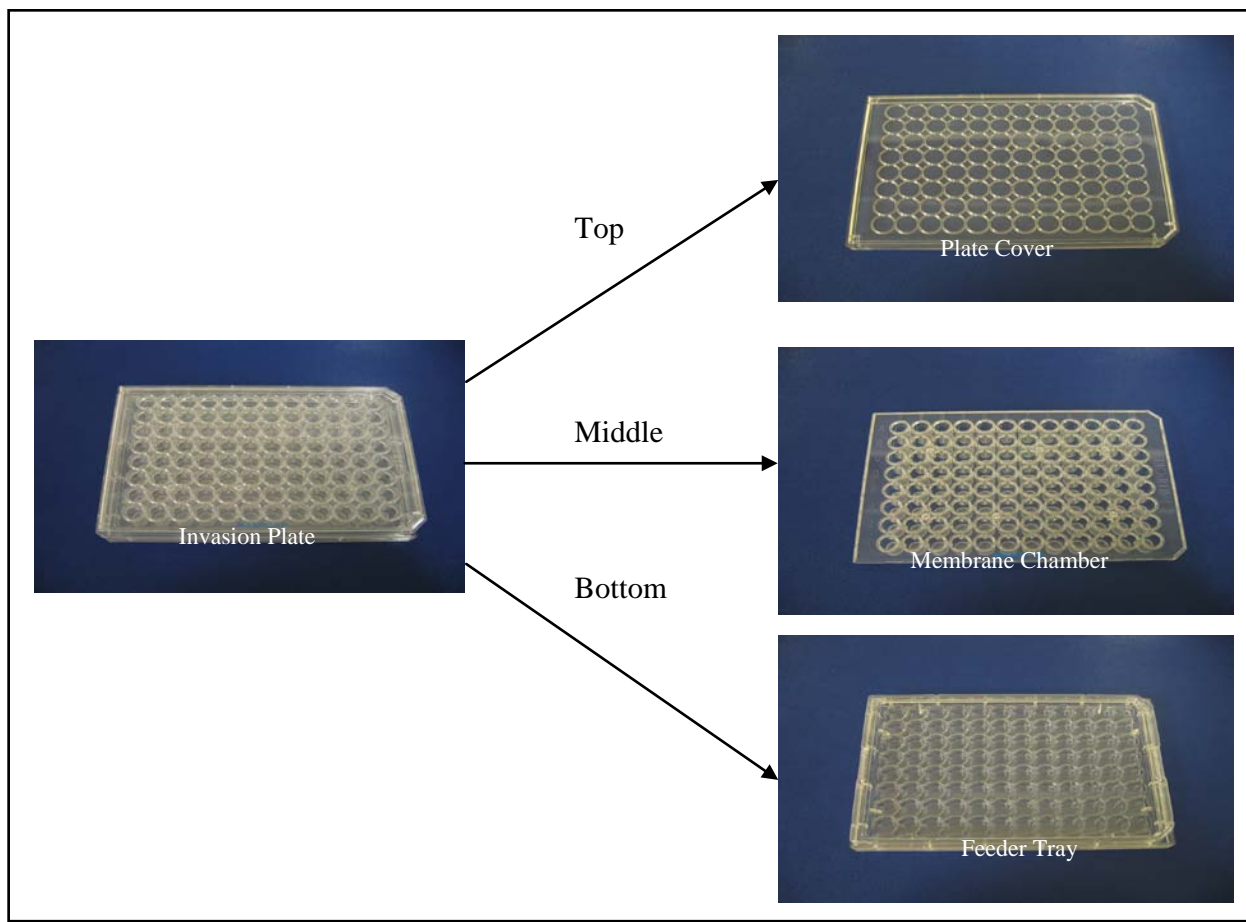
1. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
2. CBA-106-C: CytoSelect™ 96-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
3. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
4. CBA-110-COL: CytoSelect™ 24-Well Cell Invasion Assay (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, Fluorometric)
9. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
10. CBA-112-LN: CytoSelect™ 96-Well Cell Invasion Assay (Laminin I, Fluorometric)
11. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

## **Kit Components**

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<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. 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 collagen invasion plate to warm up at room temperature for 10 minutes.
2. Rehydrate the collagen layer of the membrane inserts by adding 125  $\mu\text{L}$  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.

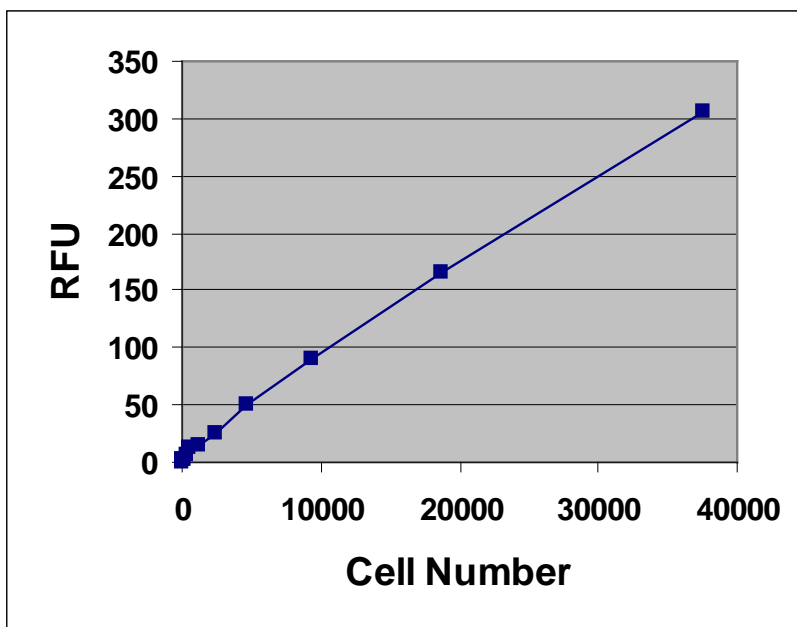
*Note: Overnight starvation may be performed prior to running the assay.*

4. Carefully remove 100  $\mu\text{L}$  of the rehydration medium (step 2) from the inserts without disturbing the collagen layer (leaving 25  $\mu\text{L}$  inside).

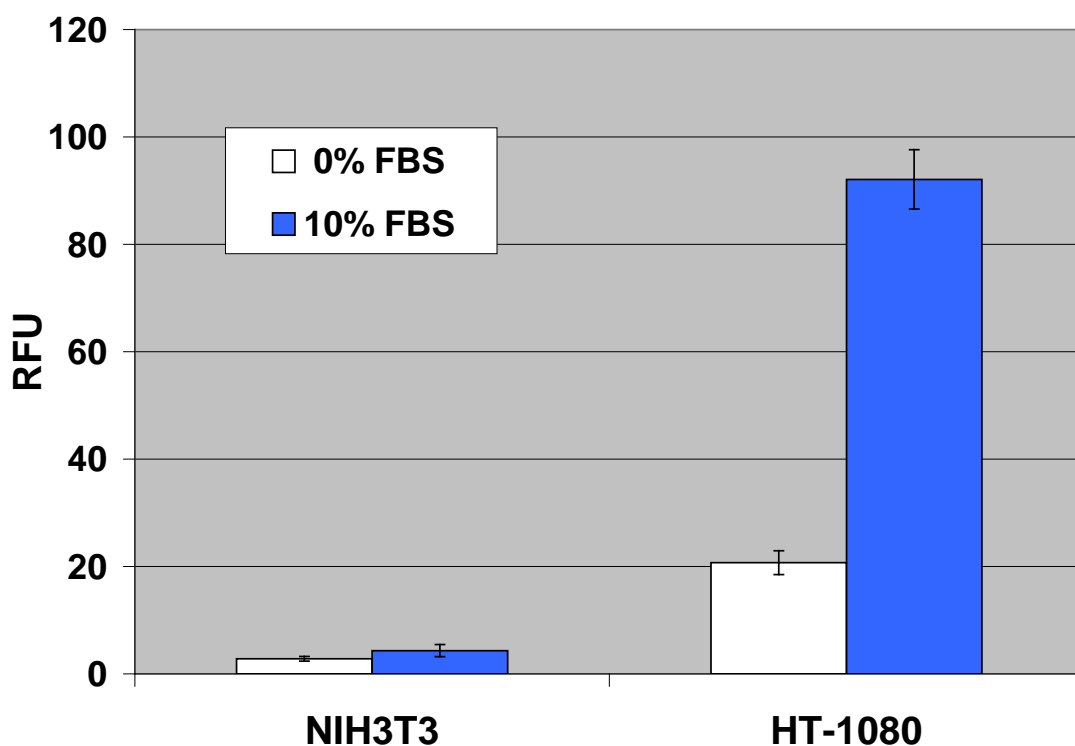
5. Under sterile conditions, separate the cover and membrane chamber from the feeder tray. Add 150  $\mu\text{L}$  of media containing 10% fetal bovine serum or desired chemoattractant(s) to the wells of the feeder tray.
6. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). **Ensure no bubbles are trapped under the membrane.**
7. Gently mix the cell suspension from step 3 and add 100  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  dye to 370  $\mu\text{L}$  of 4X Lysis Buffer).
14. Add 50  $\mu\text{L}$  of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150  $\mu\text{L}$  of Cell Detachment Solution). Incubate 20 minutes at room temperature.
15. Transfer 150  $\mu\text{L}$  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 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

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2. Montgomery, A. M. P., De Clerck, Y. A., Langley, K. E., Reisfeld, R. A., Mueller, B. M. (1993) *Cancer Res* **53**,693-700.
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## Recent Product Citations

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6. Liu, X. et al. (2013). Antiproliferative, antiinvasive, and proapoptotic activity of folate receptor  $\alpha$ -targeted liposomal doxorubicin in nonfunctional pituitary adenoma cells. *Endocrinology* **154**:1414-1423.
7. Ziemann, A. et al. (2013). CRN2 enhances the invasiveness of glioblastoma cells. *Neuro Oncology* 10.1093/neuonc/nos388.

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