Product Manual

CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Fluorometric Format)

Catalog Number

CBA-111-COL **12 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

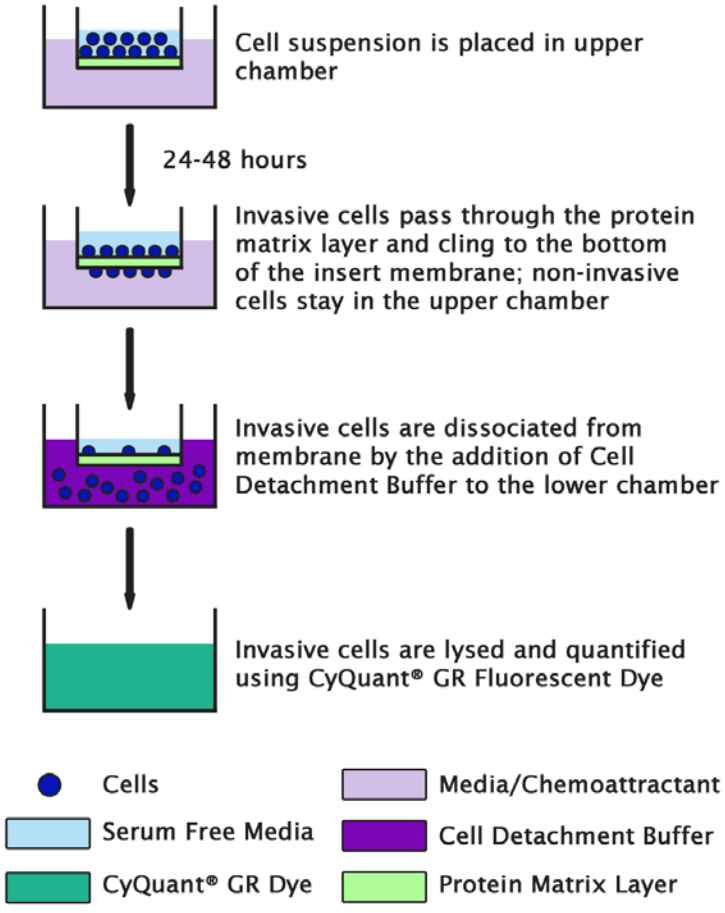
Introduction

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™ Collagen Cell Invasion Assay Kit utilizes Bovine Type I Collagen-coated inserts to assay the invasive properties of tumor cells. It contains sufficient reagents for the evaluation of 12 samples.

Assay Principle

The CytoSelect™ Collagen 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 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, Colorimetric)
6. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
7. CBA-111-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin, Fluorometric)
8. CBA-112: CytoSelect™ 96-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
9. CBA-112-COL: CytoSelect™ 96-Well Cell Invasion Assay (Collagen I, Fluorometric)
10. CBA-112-LN: CytoSelect™ 96-Well Cell Invasion Assay (Laminin, Fluorometric)
11. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

1. Collagen Invasion Chamber Plate (Part No. 111001-COL): One 24-well plate containing 12 collagen-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 $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37°C, 5% CO_2 atmosphere)
5. Light microscope
6. 96-well plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

Storage

Store all components at 4ºC until their expiration dates.

Assay Protocol

1. Under sterile conditions, allow the collagen invasion chamber plate to warm up at room temperature for 10 minutes.
2. Rehydrate the collagen layer of the cell culture inserts by adding 300 µL of warm, serum-free media to the inner compartment. Incubate at room temperature for 30 minutes.
3. Prepare a cell suspension containing 0.5-1.0 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 250 µL of rehydration medium (step 2) from the inserts without disturbing the collagen layer (leaving 50 µL inside).
5. Add 250 µL of the cell suspension solution to the inside of each insert.
6. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
7. Incubate for 12-24 hours in a cell culture incubator.
8. Carefully aspirate the media from the inside of the insert. Transfer the insert to a clean well containing 225 µL of Cell Detachment Solution. Incubate 30 minutes at 37°C.
9. Completely dislodge the invaded cells from the underside of the membrane by gently tilting the insert several times in the detachment solution. Remove and discard the insert.
10. 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 to 370 µL of 4X Lysis Buffer).
11. Add 75 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well containing cells and 225 µL of Cell Detachment Solution. Incubate 20 minutes at room temperature.
12. Transfer 200 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read 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.

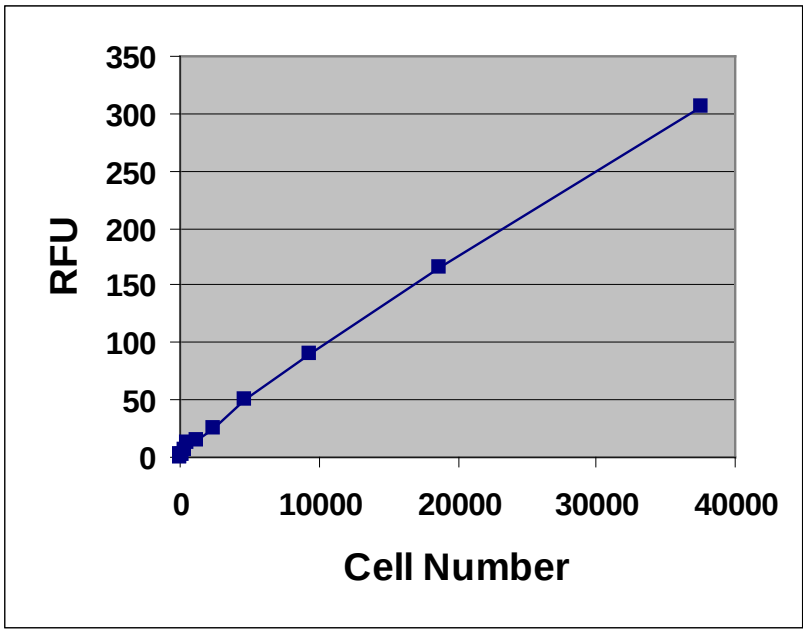


Figure 1. Quantitation of Human HT-1080. HT-1080 cell suspension was titrated 1:2 in Cell Detachment Buffer. 150 µL of each dilution was transferred to a 96-well, fluorometer plate, followed by addition of 50 µL 4X Lysis Buffer/CyQuant® GR dye solution (1:75).

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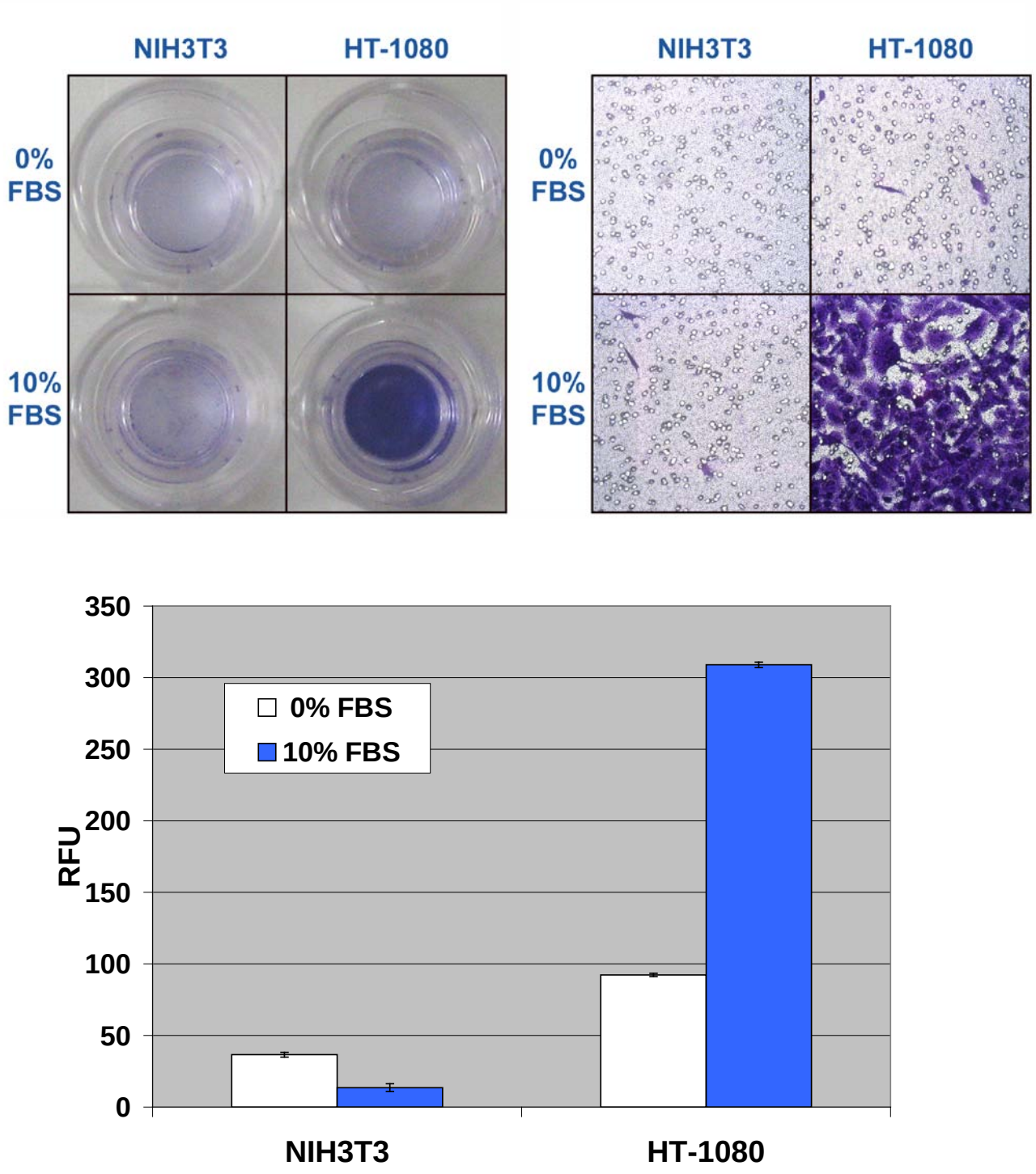

Figure 2. Human Fibrosarcoma HT-1080 Collagen Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 200,000 cells/well and allowed to invade toward FBS for 24 hrs. Invasive cells on the bottom of the invasion membrane were stained (top pictures) and quantified by CyQuant® GR dye as described in the Assay Protocol (bottom figure).

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.

Warranty

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