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

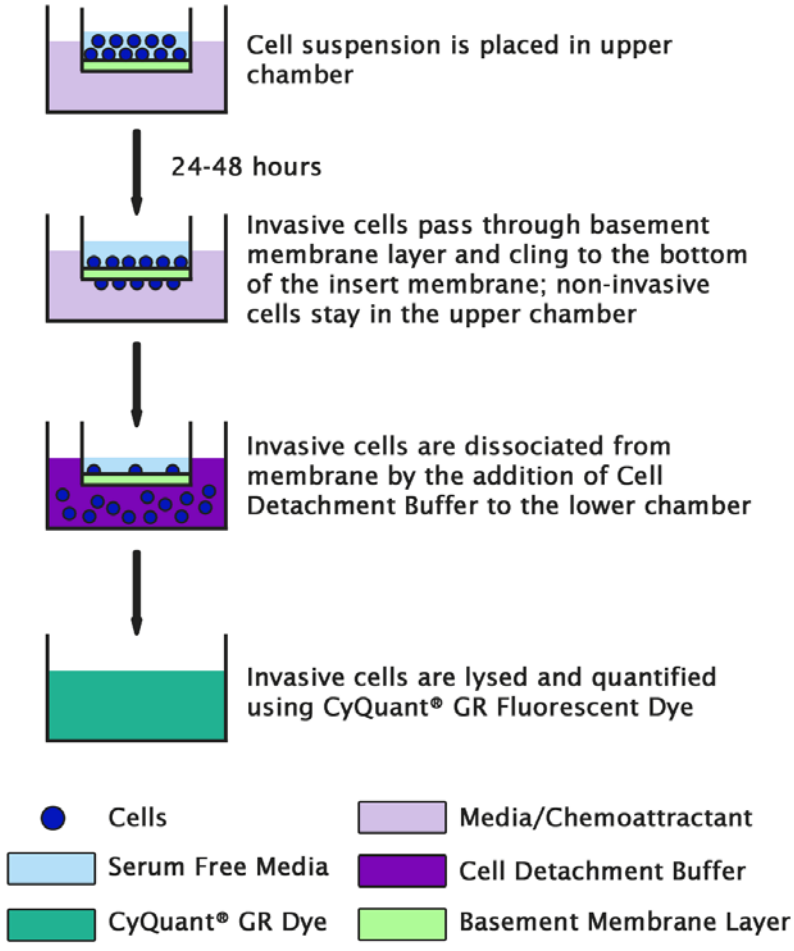
Introduction

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-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-100-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay (8µm, Colorimetric)
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, Fluorometric)
6. CBA-111-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Fluorometric)
7. CBA-111-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, 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. 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 $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Fluorescence plate reader

Storage

Store all components at 4ºC.

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 10^6 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 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. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the invasion plate.
6. Add 300 µL of the cell suspension solution to the inside of each insert.
7. Incubate for 24-48 hours at 37ºC in 5% CO_2 atmosphere.
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 37ºC.
9. 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.
10. Prepare sufficient 4X Lysis Buffer/CyQuant® GR dye solution for all samples by dilutine the dye 1:75 in 4X Lysis Buffer (for example, add 5 µL dye 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 480nm/520nm.

Example of Results

The following figures demonstrate typical invasion results 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.

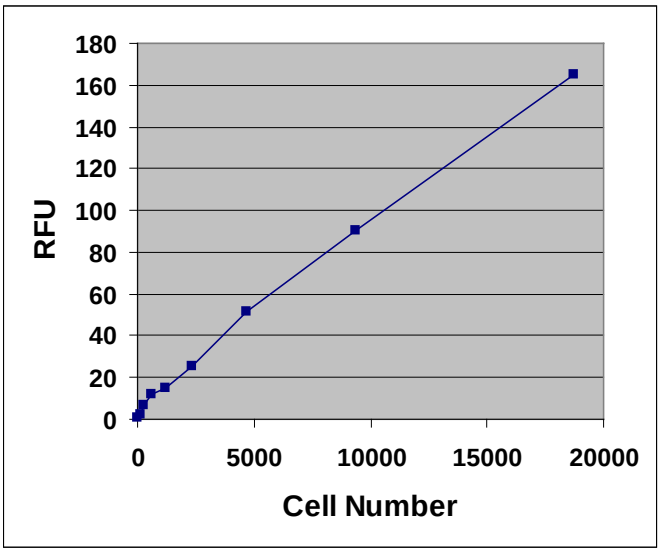


Figure 1: Quantitation of Human HT-1080. HT-1080 cell suspension was titrated in Cell Detachment Buffer; 150µL of the diluted cell suspension was mixed with 50µL of 4X Lysis Buffer/CyQuant® GR Dye (1:75).

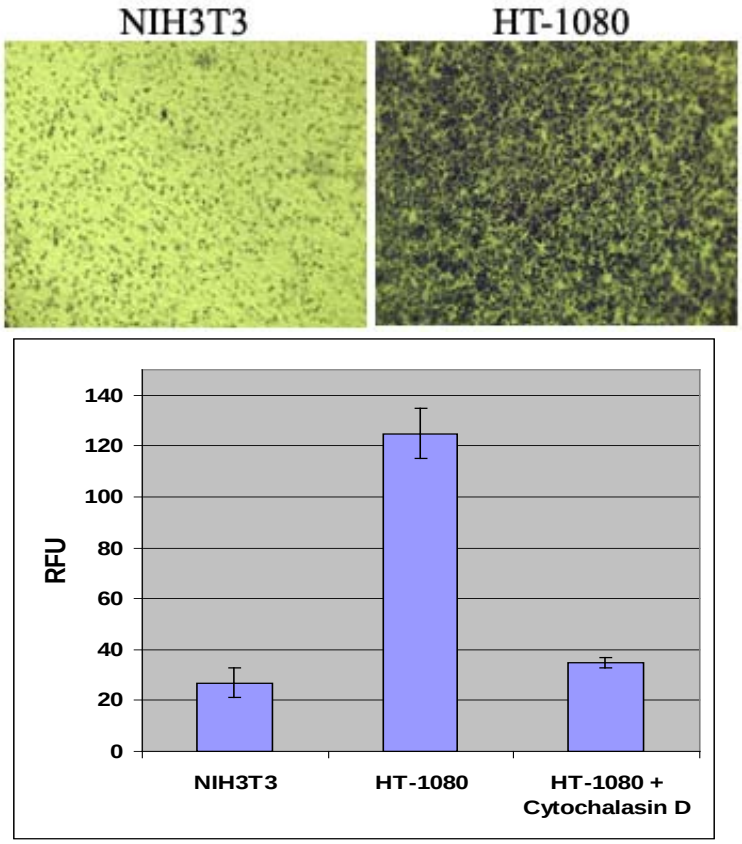


Figure 2. Human Fibrosarcoma HT-1080 Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 300,000 cells/well and allowed to invade toward 10% FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture) and quantified by CyQuant® GR Dye (bottom panel figure).

References

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Recent Product Citations

1. Paluszczak, J. et al. (2017). Lichen-derived caperatic acid and physodic acid inhibit Wnt signaling in colorectal cancer cells. *Mol Cell Biochem.* **441** (1–2):109–124.
2. Steury, M. et al. (2017). G-protein-coupled receptor kinase-2 is a critical regulator of TNFα signaling in colon epithelial cells. *Biochem. J*. **474(14)**:2301-2313.
3. Lopez-Campistrous, A. et al. (2016). PDGFRα regulates follicular cell differentiation driving treatment resistance and disease recurrence in papillary thyroid cancer. *EBioMed.* doi:10.1016/j.ebiom.2016.09.007.
4. Engel, N. et al. (2016). Antitumor evaluation of two selected Pakistani plant extracts on human bone and breast cancer cell lines. *BMC Complement Altern Med*. doi:10.1186/s12906-016-1215-9.
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6. Uddin, M. et al. (2008). Marinobufagenin inhibits proliferation and migration of cytotrophoblast and CHO cells. *Placenta* **29(3)**:266-273.
7. Ji, H. et al. (2007). LKB1 modulates lung cancer differentiation and metastasis. *Nature* **448**:807-810.

License Information

Warranty

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