Product Manual

CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric Format), Trial Size

Catalog Number

CBA-110-T	4 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

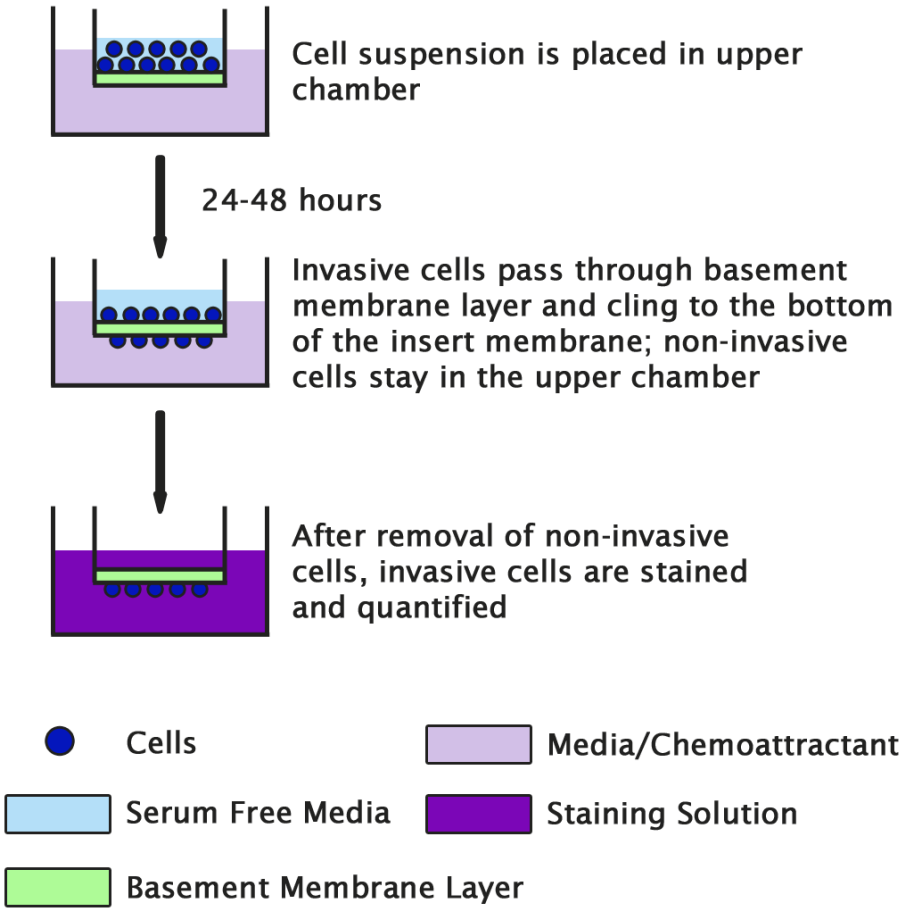
Introduction

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. This Trial Size kit contains sufficient reagents for the evaluation of 4 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, the cells are removed from the top of the membrane and the invaded cells are stained and quantified.



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-COL: CytoSelect™ 24-Well Cell Invasion Assay (Collagen I, Colorimetric)
4. CBA-110-LN: CytoSelect™ 24-Well Cell Invasion Assay (Laminin I, Colorimetric)
5. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, 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-T): One 24-well plate containing 4 ECM-coated cell culture inserts.
2. Cell Stain Solution (Part No. 11002-T): One 4 mL bottle
3. Extraction Solution (Part No. 11003-T): One 4 mL bottle
4. Cotton Swabs: (Part No. 11004) 40 each
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. Microtiter 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. Wet the ends of 2-3 cotton-tipped swabs with water, flatten the ends of the swabs by pressing them against a clean hard surface, and gently swab the interior of the inserts to remove non-invasive cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.
9. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
10. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
11. (optional) Count invasive cells with a light microscope under high magnification objective, with at least three individual fields per insert.
12. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
13. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

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.

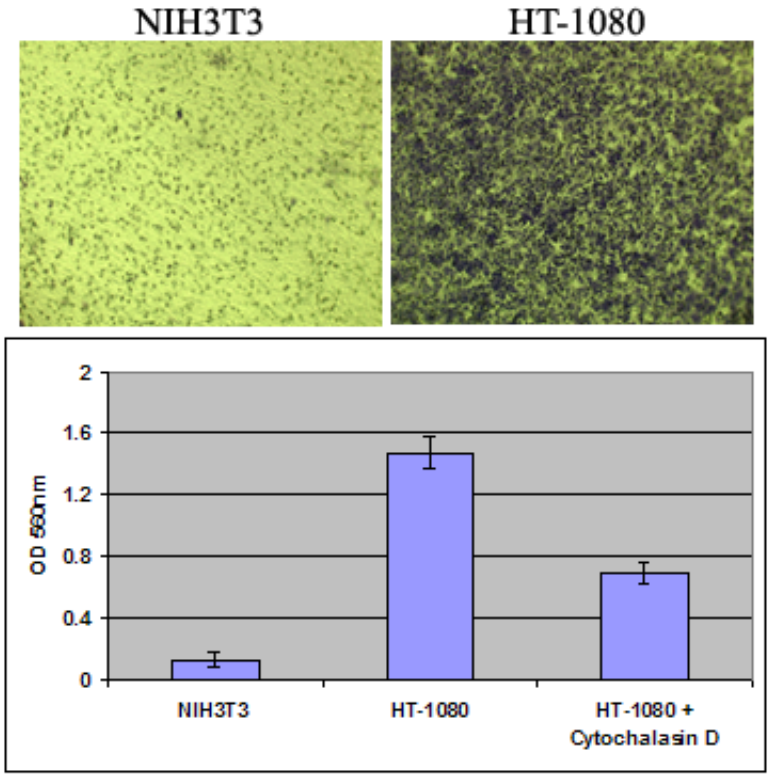


Figure 1. Human Fibrosarcoma HT-1080 Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 300,000 cells/well and allowed to invade toward 10% FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture) and quantified at OD 560nm after extraction (bottom panel figure).

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Recent Product Citations

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Please see the complete list of product citations: http://www.cellbiolabs.com/cell-invasion-assays-24-well-basement%20membrane.

Warranty

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