Product Manual

CytoSelect™ 24-Well Cell Migration and Invasion Assay (8 µm, Colorimetric Format)

Catalog Number

CBA-100-C	2 x 12 assays (12 migration + 12 invasion)
CBA-100-C-5	10 x 12 assays (60 migration + 60 invasion)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis, and arthritis. The initial response of a cell to a migration-promoting agent is to polarize and extend protrusions in the direction of the attractant; these protrusions can consist of large, broad lamellipodia or spike-like filopodia. In either case, these protrusions are driven by actin polymerization and can be stabilized by extracellular matrix (ECM) adhesion or cell-cell interactions (via transmembrane receptors).

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 Migration Assay utilizes polycarbonate membrane inserts (8 µm pore size) to assay the migratory properties of cells. The 8 µm pore size is optimal for epithelial and fibroblast cell migration. However, in the case of leukocyte chemotaxis, a smaller pore size (3 µm) is recommended. Cell Biolabs' CytoSelect™ Cell Invasion Assay utilizes basement membrane-coated inserts to assay the invasive properties of tumor cells. Each assay contains sufficient reagents for the evaluation of 12 samples.

Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-100-COL: CytoSelect™ 24-Well Cell Haptotaxis Assay (Collagen I, Colorimetric)
3. CBA-100-FN: CytoSelect™ 24-Well Cell Haptotaxis Assay (Fibronectin, Colorimetric)
4. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
5. CBA-101-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay Combo Kit (8 µm, Fluorometric)
6. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
7. CBA-106-C: CytoSelect™ 96-Well Cell Migration and Invasion Assay Combo Kit (8µm, Fluorometric)
8. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)

Kit Components

1. 24-well Migration Plate (Part No. 10001): One 24-well plate containing 12 cell culture inserts (8 µm pore size)
2. Invasion Chamber Plate (Part No. 11001): One 24-well plate containing 12 ECM-coated cell culture inserts.
3. Cell Stain Solution (Part No. 11002-C): One 20 mL bottle
4. Extraction Solution (Part No. 11003-C): One 20 mL bottle

5. Cotton Swabs (Part No. 11004): 40 each
6. Forceps: (Part No. 11005) One each

Materials Not Supplied

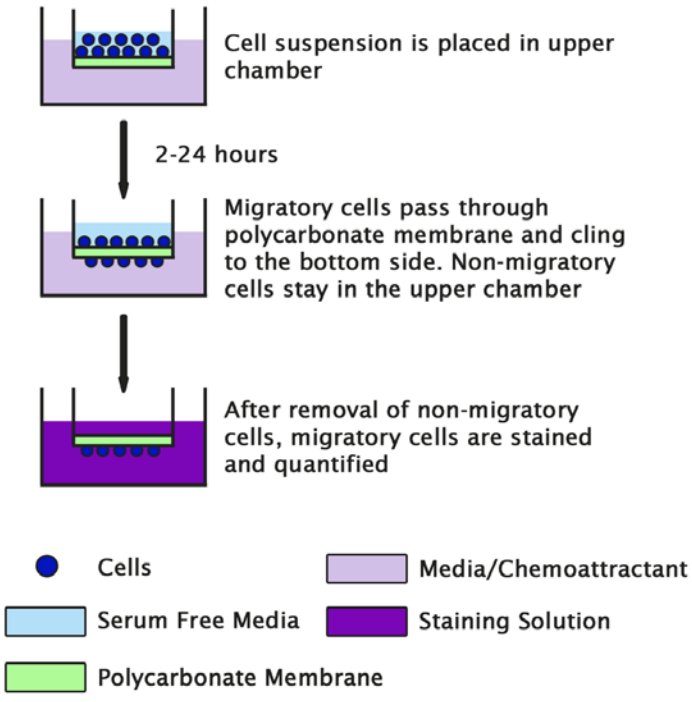
1. Migratory or invasive cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
5. Light microscope
6. 96-well microtiter plate

Storage

Store all components at 4ºC.

Cell Migration Assay Principle

The Cell Migration portion of this kit uses polycarbonate membrane inserts (8 µm pore size) in a 24-well plate. The membrane serves as a barrier to discriminate migratory cells from non-migratory cells. Migratory cells are able to extend protrusions towards chemoattractants (via actin cytoskeleton reorganization) and ultimately pass through the pores of the polycarbonate membrane. Finally, the cells are removed from the top of the membrane and the migratory cells are stained and quantified.

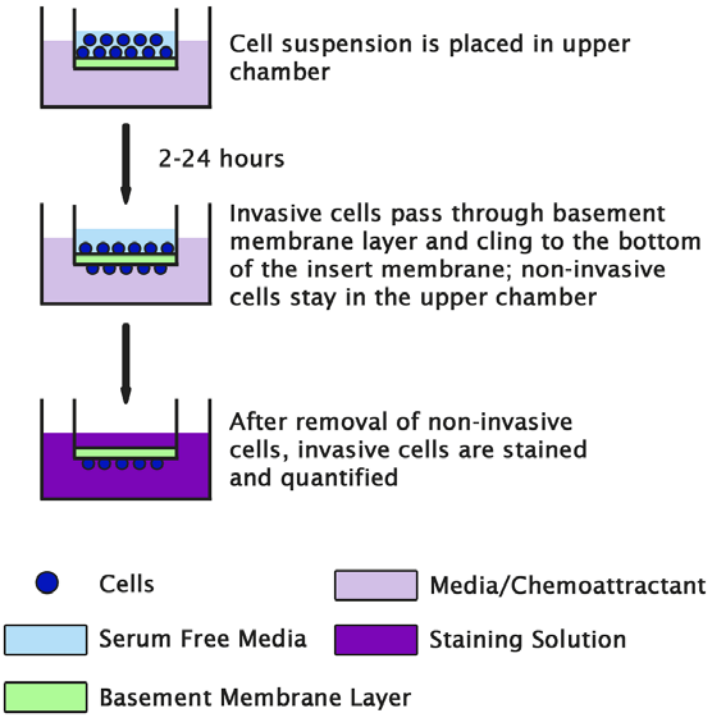


Cell Migration Assay Protocol

1. Under sterile conditions, allow the 24-well migration plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing 0.5-1.0 x 10^6 cells/ml in serum free media. Agents that inhibit or stimulate cell migration can be added directly to the cell suspension.
   Note: Overnight starvation may be performed prior to running the assay
3. Add 500 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the migration plate.
4. Add 300 µL of the cell suspension solution to the inside of each insert.
5. Incubate for 2-24 hours in a cell culture incubator.
6. Carefully aspirate the media from the inside of the insert. Wet the ends of 2-3 cotton-tipped swabs and gently swab the interior of the inserts to remove non-migratory cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.
7. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
8. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
9. (optional) Count migratory cells with a light microscope under high magnification objective, with at least three individual fields per insert.
10. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
11. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

Cell Invasion Assay Principle

The Cell Invasion Assay portion of this kit uses a 24-well plate containing polycarbonate membrane inserts (8 µm pore size); 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.



Cell Invasion 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 migration plate.
6. Add 300 µL of the cell suspension solution to the inside of each insert.
7. Incubate for 12-48 hours in a cell culture incubator.
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 with the CytoSelect™ Cell Migration and Invasion Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

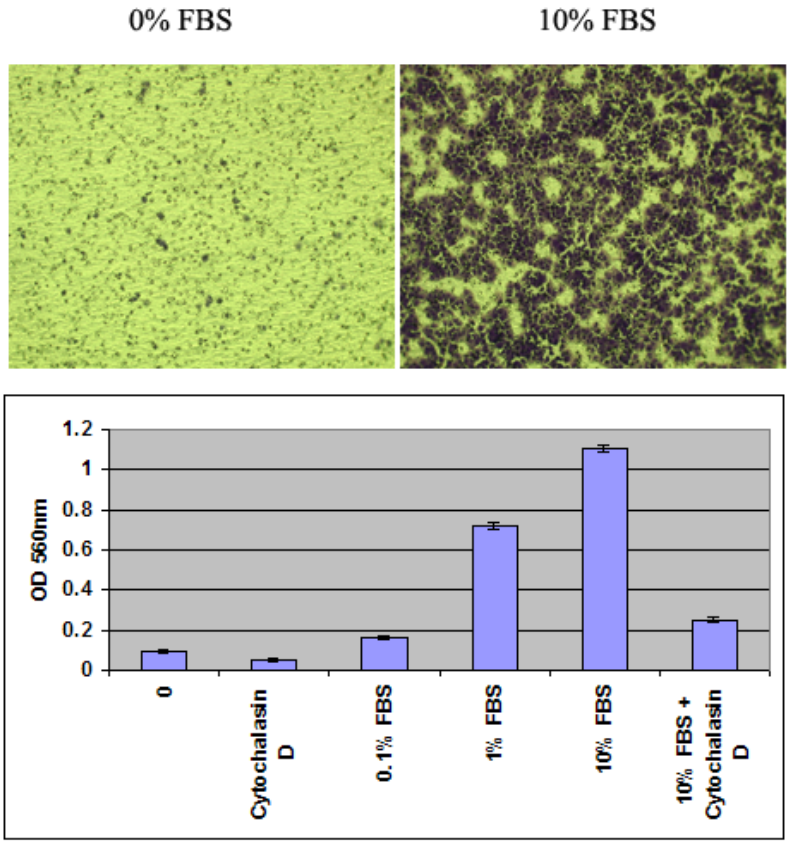


Figure 1. Human Fibrosarcoma HT-1080 Cell Migration. HT-1080 cells were seeded at 150,000 cells/well and allowed to migrate toward FBS for 4 hrs in the presence or absence of 2 µM Cytochalasin D. Migratory cells on the bottom of the polycarbonate membrane were stained (top panel picture) and quantified at OD 560nm after extraction (bottom panel figure).

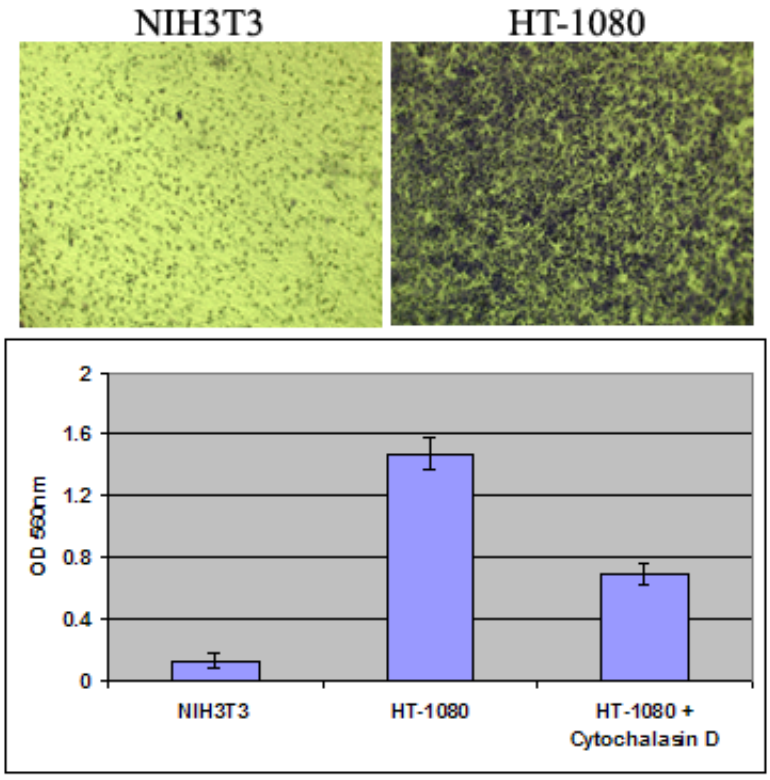


Figure 2. Human Fibrosarcoma HT-1080 Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 300,000 cells/well and allowed to invade toward FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture)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

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Recent Product Citations

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com