Product Manual

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

Catalog Number

CBA-101-C	2 x 12 assays (12 migration + 12 invasion)

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

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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 and Invasion Assay utilize polycarbonate membrane inserts (8 µm pore size) or basement membrane-coated inserts to assay the migratory or invasive properties of cells. The kit does not require you to prelabel the cells with Calcein AM or remove non-migratory or non-invasive cells (i.e. cotton swabbing). Any migratory or invasive cells are first dissociated from the membrane, then lysed and detected by the patented CyQuant® GR Dye (Invitrogen).

TheCytoSelect™ Cell Migration and Invasive Assay Kit provides a robust system for the quantitative determination of cell migration. Each assay contains sufficient reagents for the evaluation of 12 samples. 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.

Related Products

1. CBA-100-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay Combo Kit (8 µm, Colorimetric)
2. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
3. CBA-101-COL: CytoSelect™ 24-Well Cell Haptotaxis Assay (Collagen I, Fluorometric)
4. CBA-101-FN: CytoSelect™ 24-Well Cell Haptotaxis Assay (Fibronectin, Fluorometric)
5. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
6. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
7. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
8. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
9. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
10. CBA-126: Radius™ 96-Well Cell Migration Assay (Microscopy)
11. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

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 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. 105101): One 50 µL tube
6. Forceps (Part No. 11005): One each

Materials Not Supplied

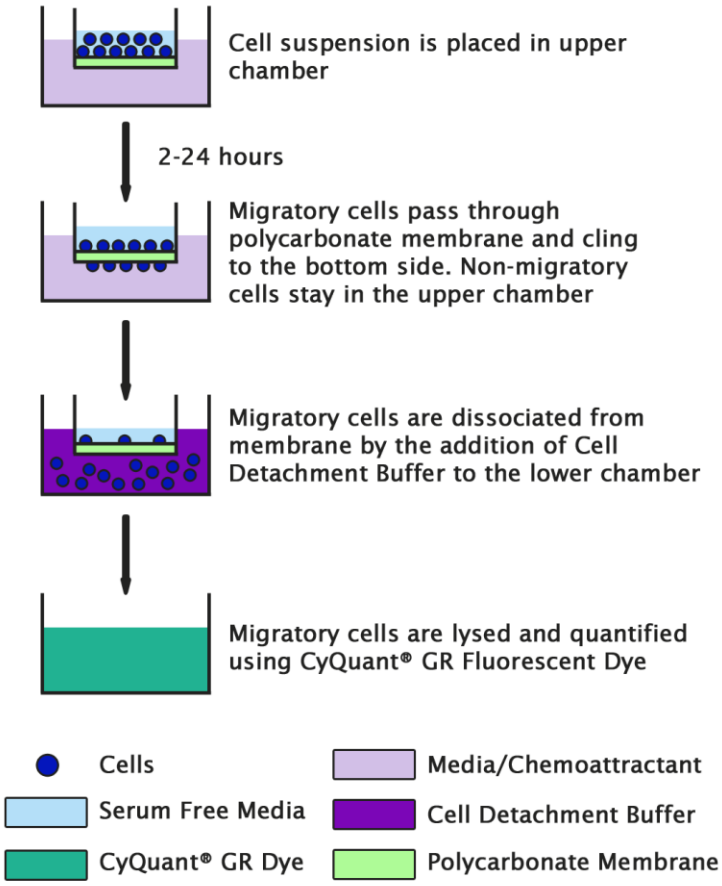
1. Migratory or invasive cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
5. Light microscope
6. 96-well plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

Storage

Store all components at 4ºC until their expiration dates.

Cell Migration Assay Principle

The CytoSelect™ Cell Migration Assay Kit contains 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. These migratory cells are then dissociated from the membrane and subsequently detected by the patented CyQuant® GR Dye (Invitrogen).



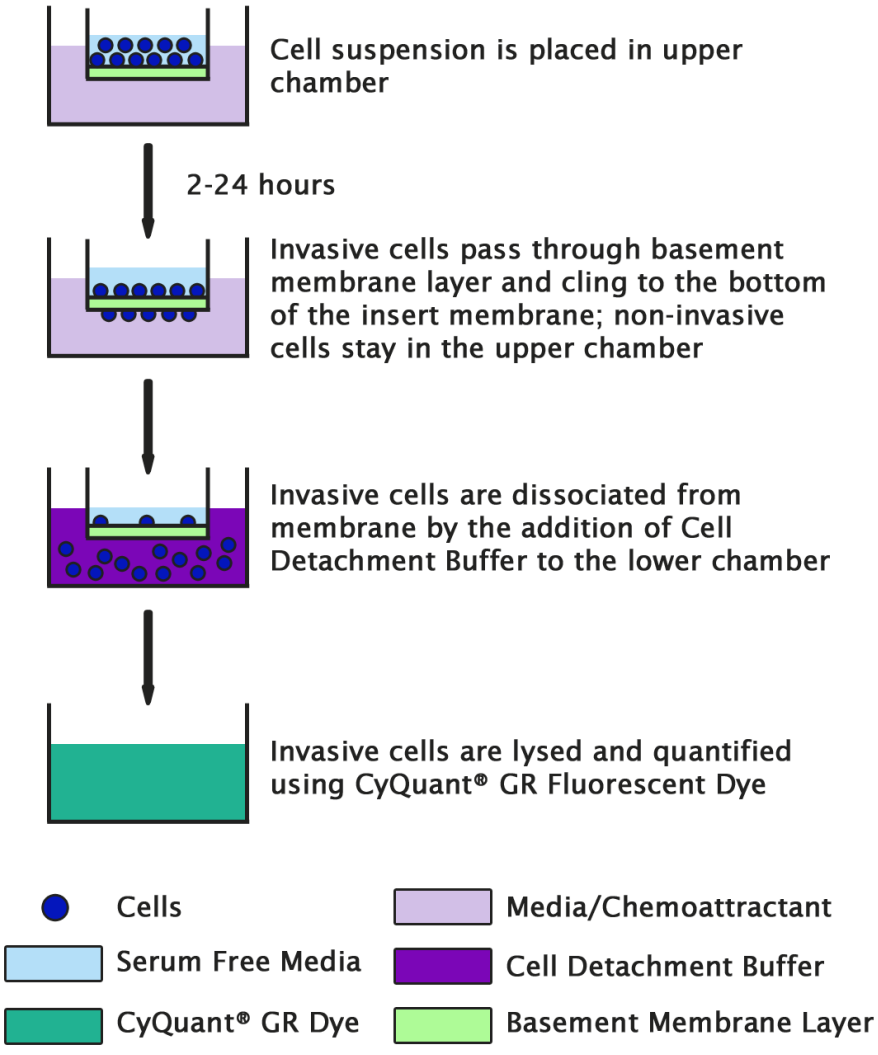
Cell Migration Assay Protocol

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. Transfer the insert to a clean well containing 225 µL of Cell Detachment Solution. Incubate 30 minutes at 37ºC.
7. 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.
8. 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 dye to 370 µL of 4X Lysis Buffer).

9. 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.
10. Transfer 200 µL of the mixture a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480 nm/520 nm.

Cell Invasion 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 invaded cells are dissociated from the membrane and subsequently detected with CyQuant® GR Dye.



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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. 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 diluting 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 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™ Cell Migration and 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.

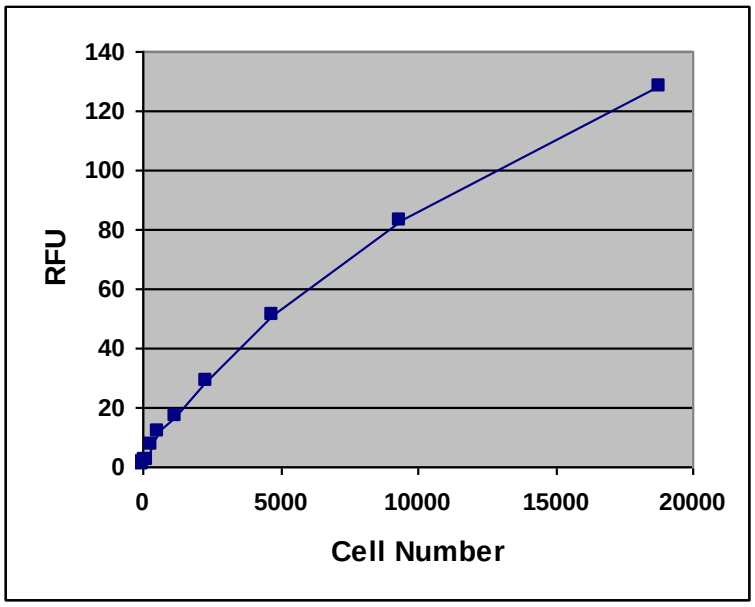


Figure 1: **Quantitation of Human HT-1080**. HT-1080 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150µL cell suspension was mixed with 50µL of 4X Lysis Buffer/dye).

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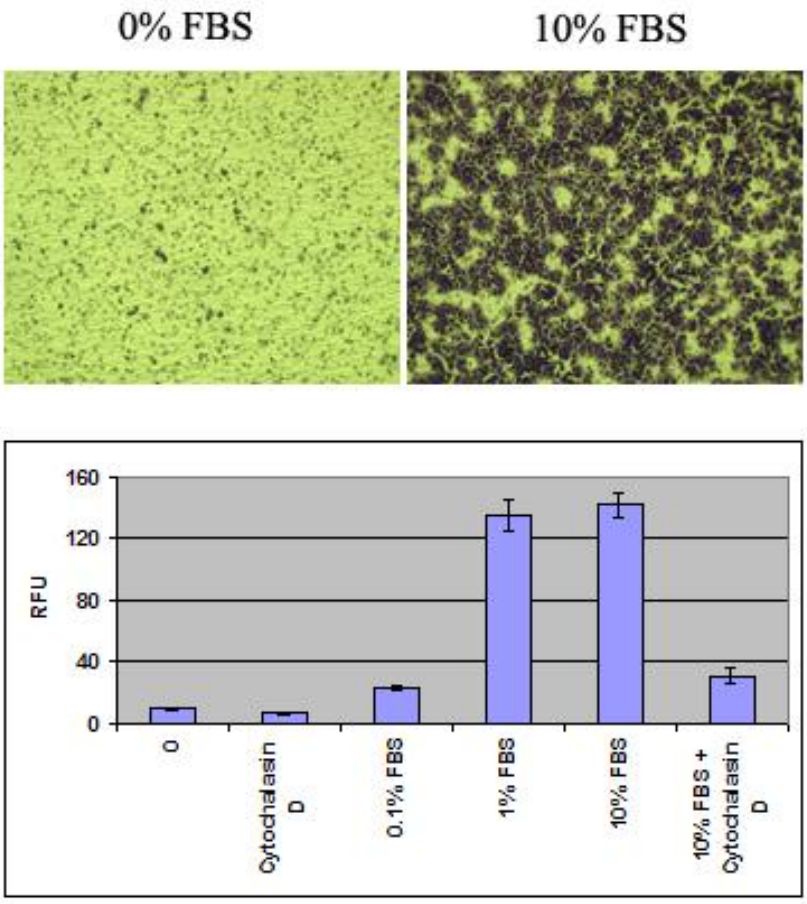


Figure 2. Human Fibrosarcoma HT-1080 Cell Migration. HT-1080 was seeded at 30,000 cells/well and allowed to migrate toward FBS for 4 hrs in the presence or absence of 2 µM Cytochalasin D. Migratory cells on the bottom of the polycarbonate membrane were stained (top panel picture) and quantified by CyQuant® GR Dye as described in the Assay Protocol.

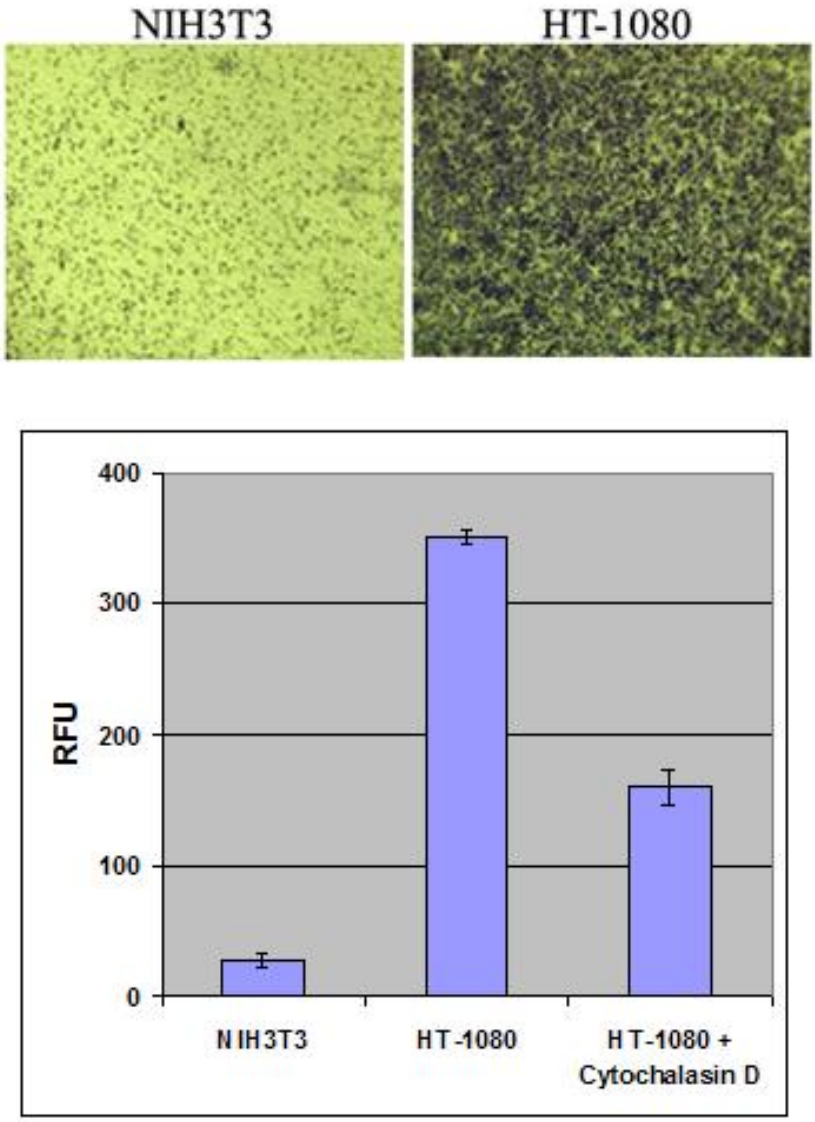


Figure 3. Human Fibrosarcoma HT-1080 Cell Invasion. HT-1080 and NIH3T3 (negative control) were seeded at 300,000 cells/well and allowed to invade toward FBS for 24 hrs in the presence or absence of 2 µM Cytochalasin D. Invasive cells on the bottom of the invasion membrane were stained (top panel picture) and quantified by CyQuant® GR Dye as described in Assay Protocol (bottom panel figure).

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Recent Product Citations

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