Product Manual

CytoSelect™ 96- Well Cell Migration Assay (3 µm, Fluorometric Format)

Catalog Number

CBA- 104	96 assays
CBA- 104- 5	5 x 96 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

Introduction

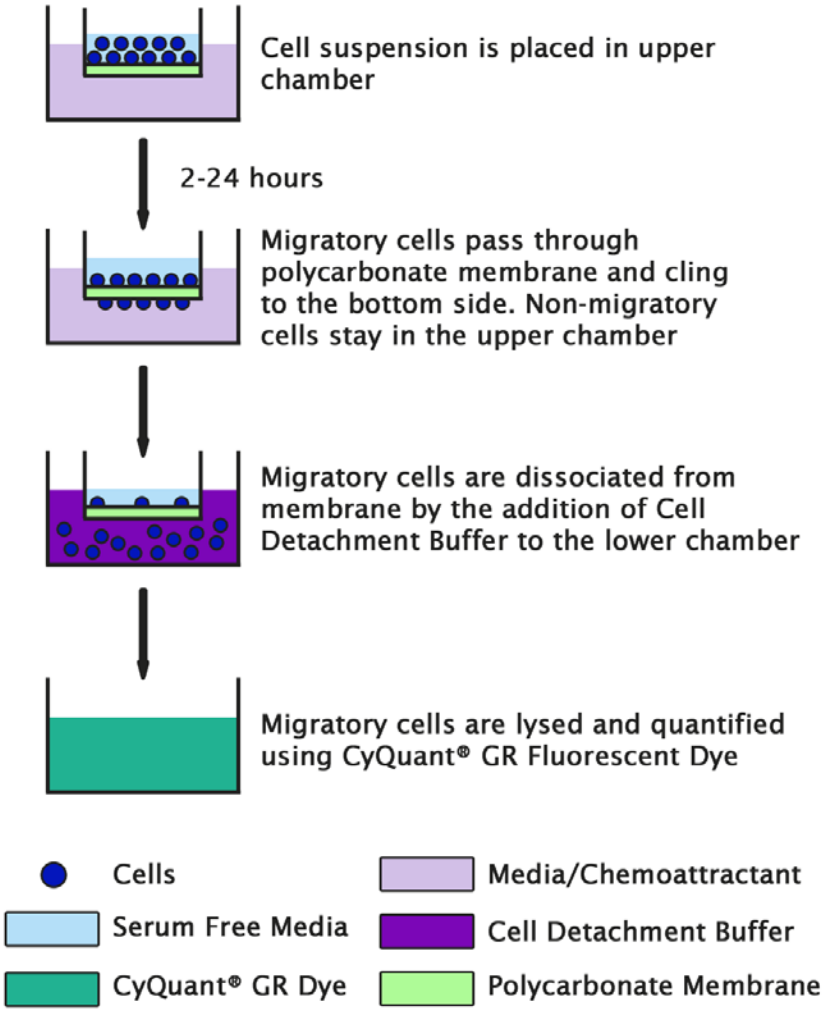
Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis, and arthritis. The initial response of a cell to a migration-promoting 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.

Cell Biolabs CytoSelect™ 96-well Cell Migration Assay Kit utilizes a polycarbonate membrane plate (3 µm pore size) to assay the migratory properties of cells. The kit does not require you to prelabel the cells with Calcein AM or remove non-migratory cells (i.e. cotton swabbing). Any migratory cells are first dissociated from the membrane, then lysed and detected with CyQuant® GR Dye.

Cell Biolabs CytoSelect™ 96-well Cell Migration Assay Kit provides a robust system for the quantitative determination of cell migration. The kit contains sufficient reagents for the evaluation of 96 samples. The 3 µm pore size is optimal for leukocyte cell migration. However, in the case of epithelial or fibroblast chemotaxis, a larger pore size (8 µm) is recommended.

The CytoSelect™ Cell Migration Assay Kit contains a polycarbonate membrane chamber (3 µm pore size) in a 96-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 with CyQuant® GR Dye.

Assay Principle



Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
3. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
4. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
5. CBA-105: CytoSelect™ 96-Well Cell Migration Assay (5µm, Fluorometric)
6. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
7. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)
8. CBA-120: CytoSelect™ 24-Well Wound Healing Assay (Light Microscopy)
9. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy
10. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components

1. 96-well Cell Migration Plate (Part No. 10401): One sterile 96-well plate (see Figure 1 for components)
2. 96-well Cell Harvesting Tray (Part No. 10402): One 96-well tray
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. 10105): One 75 µL tube

Materials Not Supplied

1. Migratory 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. FBS or desired chemoattractant
5. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
6. Light microscope
7. 96-well plate suitable for a fluorescence plate reader
8. Fluorescence plate reader

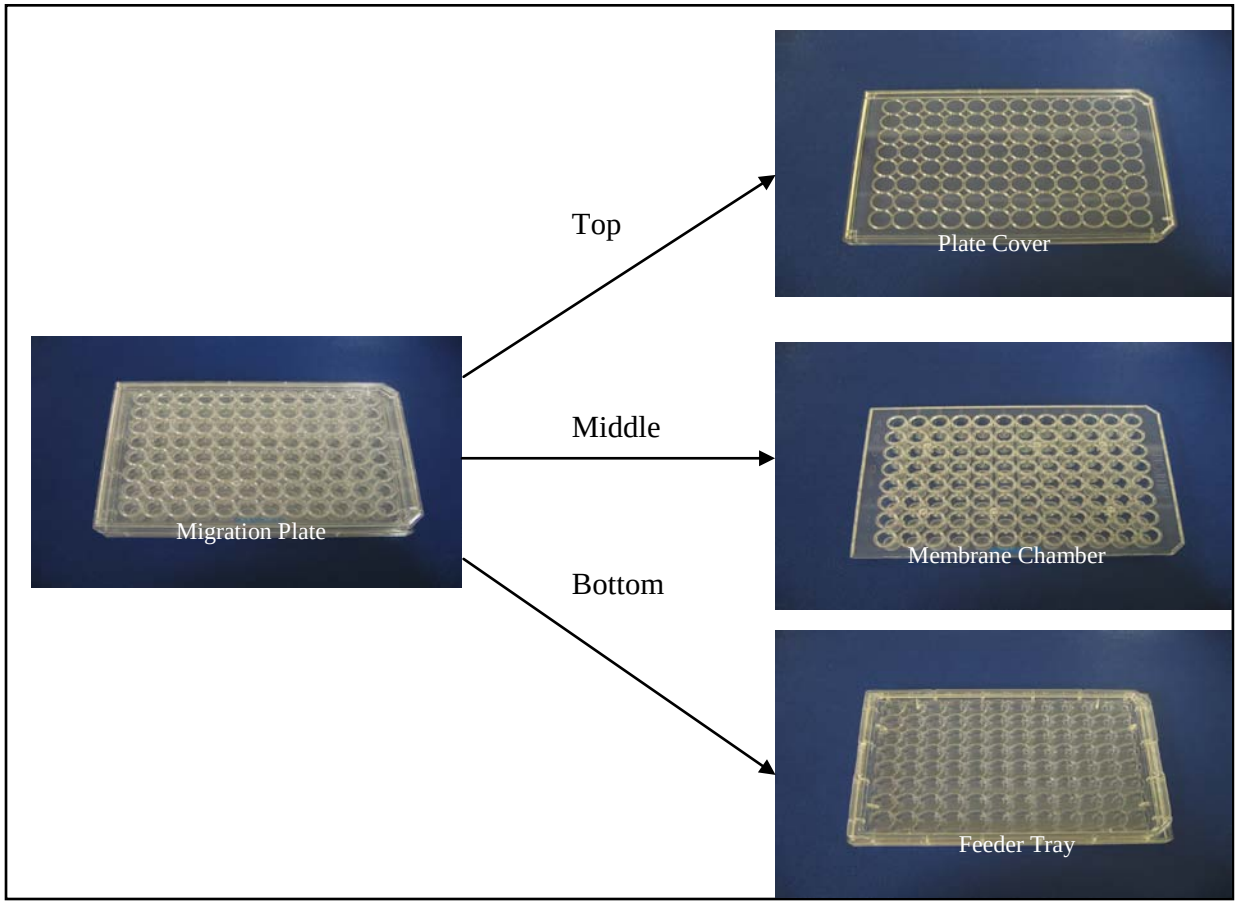


Figure 1: **Components of the 96-well Cell Migration Plate.**

Storage

Store all components at 4ºC.

Assay Protocol

1. Allow the 96-well Migration Plate to warm up at room temperature for 10 minutes.
2. Prepare a cell suspension containing 0.5-5.0 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. Under sterile conditions, separate the cover and membrane chamber from the 96-well Migration Plate.
4. Add 150 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the wells of the feeder tray.
5. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). **Ensure no bubbles are trapped under the membrane.**

6. Gently mix the cell suspension (without chemoattractant) from step 2 and add 100 µL to the membrane chamber.
7. Finally, cover the plate and transfer to a cell culture incubator for 2-24 hours.
8. Just prior to the end of the incubation, pipette 150 µL of prewarmed Cell Detachment Solution into wells of the clean, 96-Well Cell Harvesting Tray (provided).
9. Carefully remove the 96-well Migration Plate from the incubator. Separate the membrane chamber from the feeder tray.
   Note: Retain the feeder tray for step 12.
10. Remove the cells/media from the top side of the membrane chamber by aspirating or inverting. Place the membrane chamber into the Cell Harvesting Tray containing 150 µL of Cell Detachment Solution (step 8). Incubate 30 minutes at 37ºC.
11. Completely dislodge the cells from the underside of the membrane by gently tilting the membrane chamber several times in the Cell Detachment Solution.
12. In a clean 96-well plate (not provided), combine 75 µL of media from the feeder tray (step 9) with 75 µL of the detachment solution (step 11).
13. 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).
14. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of Cell Detachment Solution). Incubate 20 minutes at room temperature.
15. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read the fluorescence with a fluorescence plate reader at 480 nm/520 nm.

Example of Results

The following figures demonstrate typical with the CytoSelect™ Cell Migration 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.

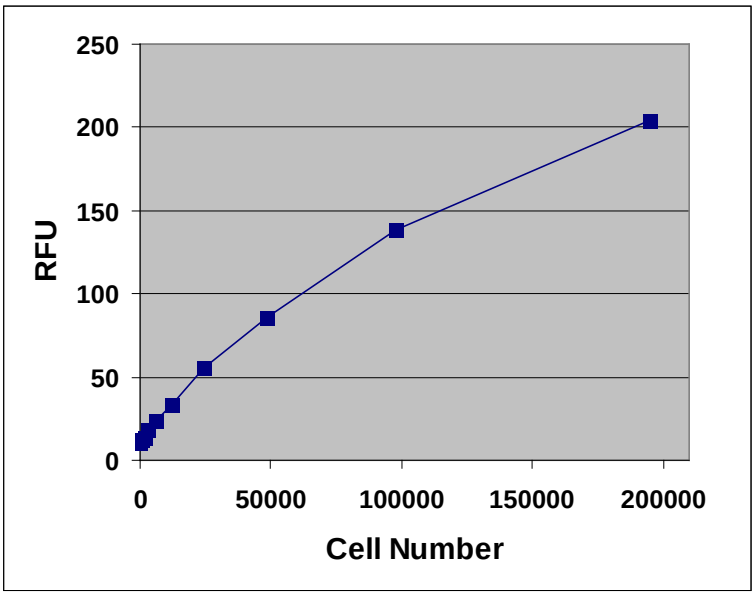


Figure 2: **Quantitation of HL-60**. HL-60 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150 µL cell suspension was mixed with 50 µL of 4X Lysis Buffer/dye).

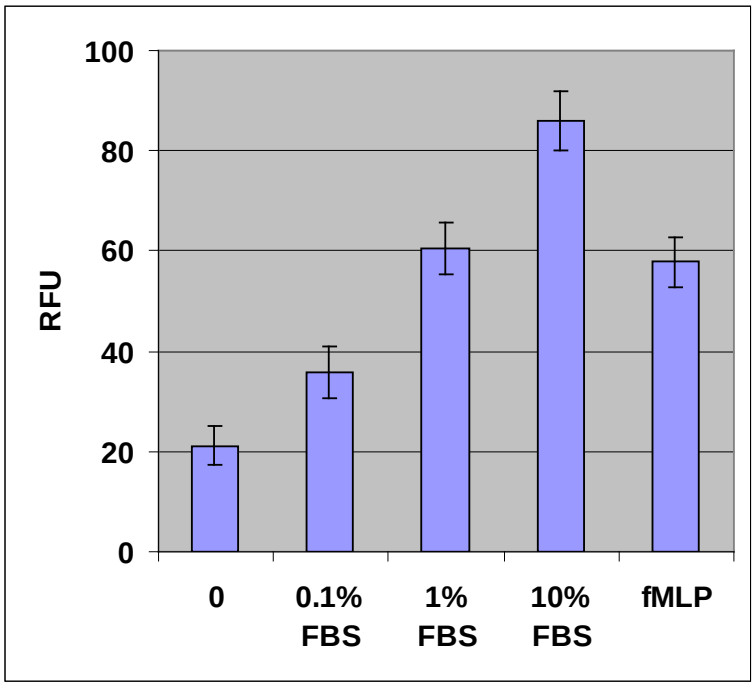


Figure 3. HL-60 Chemotaxis. HL-60 cells were allowed to migrate toward FBS or fLMP (200 nM) for 1 hr, 250,000 cells were used in each assay. Migratory cells were quantified by CyQuant® GR Dye as described in the Assay Protocol.

References

1. Ridley AJ, Schwartz MA, Burridge K, Firtel RA, Ginsberg MH, Borisy G, Parsons JT, Horwitz AR. (2003) *Science* **302**, 1704-9.
2. Horwitz R, Webb D. (2003) *Curr Biol.* **13**, R756-9.
3. Lauffenburger DA, Horwitz AF. (1996) *Cell* **84**, 359-369.

Recent Product Citations

1. Phan, T.X. et al. (2016). Intrinsic photosensitivity enhances motility of T lymphocytes. *Sci. Rep.* **6**:39479.
2. Jiang, W. et al. (2016). Infiltration of CCR2+ Ly6Chigh proinflammatory monocytes and neutrophils into the central nervous system is modulated by nicotinic acetylcholine receptors in a model of multiple sclerosis. *J Immunol.* **196**:2095-2108.
3. Kitano, K. et al. (2014). Rho-kinase activation in leukocytes plays a pivotal role in myocardial ischemia/reperfusion injury. *PLoS One.* **9**:e92242.
4. Li, X. et al. (2011). Kaposi's Sarcoma-associated Herpesvirus-encoded latency-associated nuclear antigen reduces Interleukin-8 expression in endothelial cells and impairs neutrophil chemotaxis by degrading nuclear p65. *J. Virol.* **85**:8606-8615.
5. Chatterjee, S. et al. (2009). Site-specific carboxypeptidase B1 tyrosine nitration and pathophysiological implications following its physical association with nitric oxide synthase-3 in experimental sepsis. *J. Immunol.* **183**:4055-4066.

License Information

Warranty

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