

**NOTE: Revision to
Assay Protocol**

Product Manual

CytoSelect™ 24-Well Cell Migration Assay (3 μ m, Fluorometric Format)

Catalog Number

| | |
|------------------|----------------------|
| CBA-103 | 12 assays |
| CBA-103-5 | 5 x 12 assays |

**FOR RESEARCH USE ONLY
Not for use in diagnostic procedures**

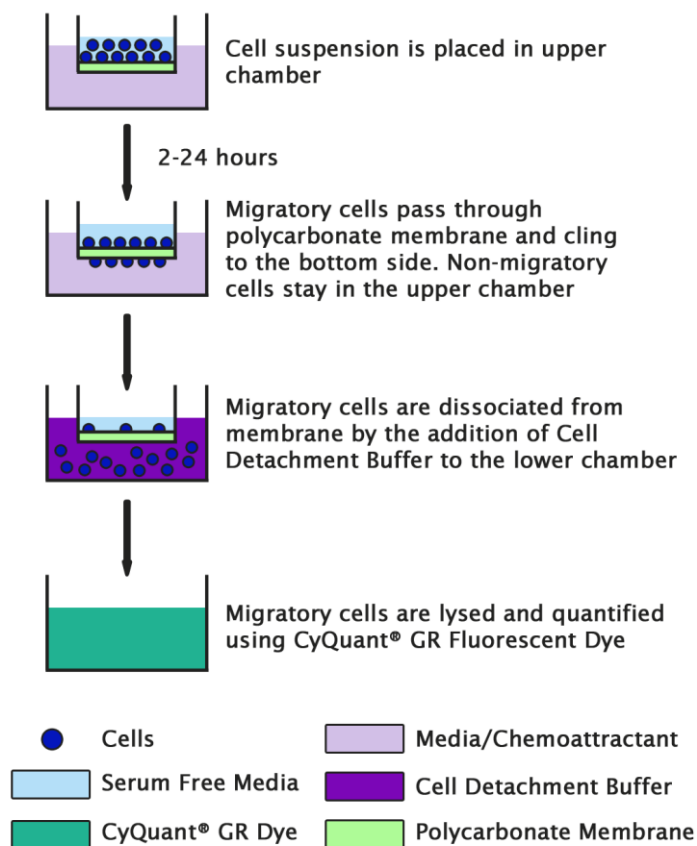


CELL BIOLABS, INC.
Creating Solutions for Life Science Research

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, 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).

Cell Biolabs CytoSelect™ Cell Migration Assay Kit utilizes polycarbonate membrane inserts (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 by the patented CyQuant® GR Dye (Invitrogen). This kit also provides a robust system for the quantitative determination of cell migration. The kit contains sufficient reagents for the evaluation of 12 samples. The 3 µm pore size is optimal for leukocyte chemotaxis. However, in the case of epithelial and fibroblast, a larger pore size (8 µm) is recommended. Lastly, this kit contains polycarbonate membrane inserts (3 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).



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-104: CytoSelect™ 96-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. 24-well Migration Plate (Part No. 10301): One 24-well plate containing 12 cell culture inserts (3 µm pore size)
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. Migratory cell lines
2. Cell culture medium
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM CaCl₂ and 2 mM MgCl₂
4. Cell culture incubator (37°C, 5% CO₂ atmosphere)
5. Light microscope
6. 96-well plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

Storage

Store all components at 4°C.

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 $0.5-5.0 \times 10^6$ cells/ml in serum free media. Agents that inhibit or stimulate cell migration can be added directly to the cell suspension.
3. Add 500 μ L of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the migration plate.
4. Add 100 μ L of the cell suspension solution to the inside of each insert.
5. Incubate for 1-24 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 200 μ L of Cell Detachment Solution. Incubate 30 minutes at 37°C.
Note: Retain the medium in the 24-well migration plate that contains chemoattractant(s) and cells that migrated through the membrane and into the medium.
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. Transfer 400 μ L of the 500 μ L medium solution containing migratory cells (step 5) to the well that contains 200 μ L of Cell Detachment Solution for the same migration assay sample (step 7). Mix well, transfer 180 μ L of the mixture to a 96-well plate.
Note: This step combines cells that migrated through the membrane and into the medium, and migratory cells detached from the bottom side of the membrane by Cell Detachment Solution.
9. 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 μ L dye to 370 μ L of 4X Lysis Buffer).
10. Add 60 μ L of 4X Lysis Buffer/CyQuant® GR dye solution to each well of the 96-well plate containing migratory cells. Incubate 20 minutes at room temperature.
11. Transfer 200 μ L 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 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.

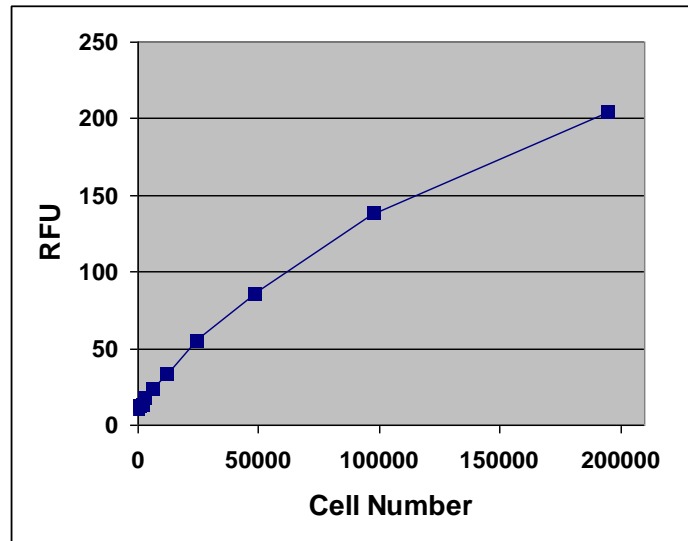


Figure 1: Quantitation of Human promyelocytic cell line HL-60. HL-60 cells were titrated in Cell Detachment Solution, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150 μ L cell suspension was mixed with 50 μ L of 4X Lysis Buffer/dye).

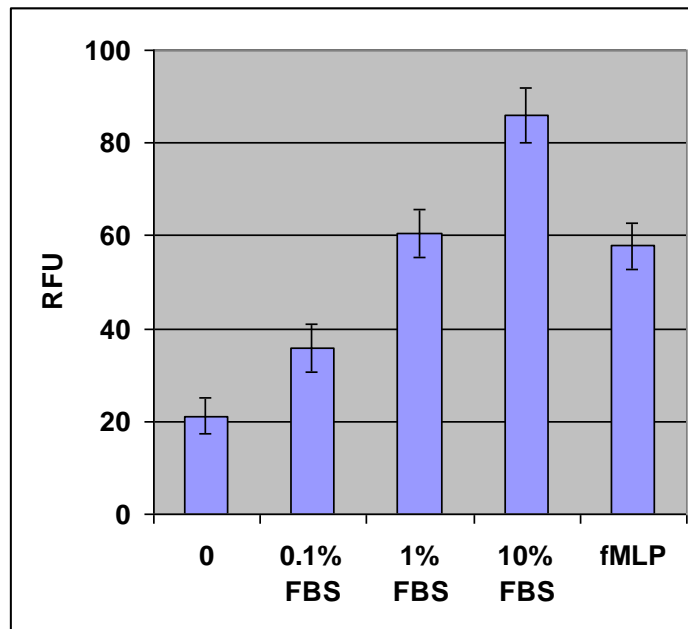


Figure 2. HL-60 Chemotaxis. 250,000 cells/well of HL-60 were allowed to migrate toward FBS or fMLP (100 nM) for 1 hr. Migratory cells were quantified by CyQuant® GR Dye as described in Assay Protocol.

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. Ikeda, M. et al. (2022). The mouse homolog of the mutant WASp responsible for human X-linked neutropenia renders granulopoiesis ineffective. *Biochem Biophys Res Commun.* **622**:177-183. doi: 10.1016/j.bbrc.2022.07.037.
2. Ali, M.S. et al. (2021). Aronia melanocarpa Extract Fermented by Lactobacillus plantarum EJ2014 Modulates Immune Response in Mice. *Antioxidants (Basel).* **10**(8):1276. doi: 10.3390/antiox10081276.
3. Chung, N.P.Y. et al. (2021). HIV induces airway basal progenitor cells to adopt an inflammatory phenotype. *Sci Rep.* **11**(1):3988. doi: 10.1038/s41598-021-82143-1.
4. Akhtar, N.M.Y. et al. (2019). Standardized ethanol extract, essential oil and zerumbone of Zingiber zerumbet rhizome suppress phagocytic activity of human neutrophils. *BMC Complement Altern Med.* **19**(1):331. doi: 10.1186/s12906-019-2748-5.
5. Domingues, C.C. et al. (2019). Reassessing the effects of continuous positive airway pressure (CPAP) on arterial stiffness and peripheral blood derived CD34+ progenitor cells in subjects with sleep apnea. *Stem Cell Res Ther.* **10**(1):147. doi: 10.1186/s13287-019-1251-8.
6. Kulkarni, U. et al. (2019). Excessive neutrophil levels in the lung underlie the age-associated increase in influenza mortality. *Mucosal Immunol.* **12**(2):545-554. doi: 10.1038/s41385-018-0115-3.
7. Ghazalee, N.S. et al. (2019). Immunosuppressive effects of the standardized extract of Zingiber zerumbet on innate immune responses in Wistar rats. *Phytother Res.* doi: 10.1002/ptr.6285.
8. Hussain, M. et al. (2019). CRTH2 antagonist, CT-133, effectively alleviates cigarette smoke-induced acute lung injury. *Life Sci.* **216**:156-167. doi: 10.1016/j.lfs.2018.11.039.
9. Słoniecka, M. et al. (2015). Substance P enhances keratocyte migration and neutrophil recruitment through interleukin-8. *Mol Pharmacol.* doi:10.1124/mol.115.101014.
10. Ilangkovan, M. et al. (2015). Immunosuppressive effects of the standardized extract of Phyllanthus amarus on cellular immune responses in Wistar-Kyoto rats. *Drug Des Devel Ther.* **9**:4917.
11. Roh, K. B. et al. (2014). Synephrine inhibits eotaxin-1 expression via the STAT6 signaling pathway. *Molecules.* **19**:11883-11895.
12. Verma, S. et al. (2011). Selenoprotein K knockout mice exhibit deficient calcium flux in immune cells and impaired immune responses. *J. Immunol.* **186**:2127-2137.

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