NOTE: Revision to Assay Protocol

Product Manual

CytoSelect™ 24-Well Cell Migration Assay (3 µm, Fluorometric Format), Trial Size

Catalog Number

CBA-103-T **4 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

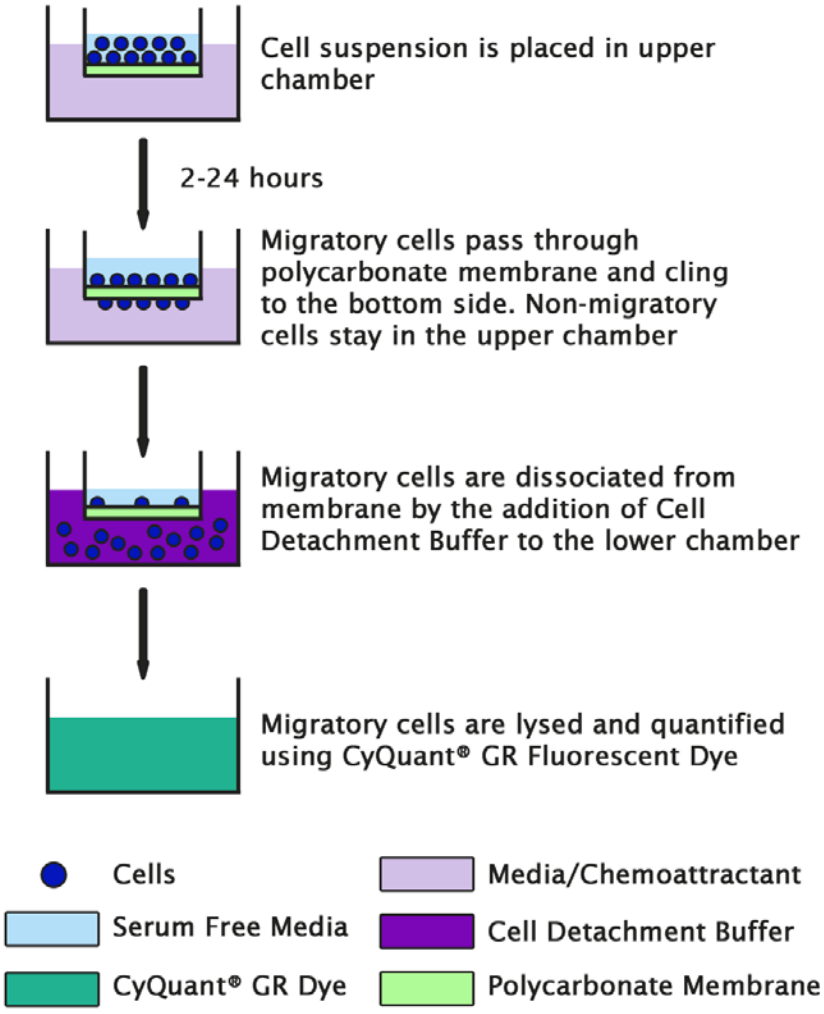
Cell migration is a highly integrated, multistep process that orchestrates embryonic morphogenesis, tissue repair and regeneration. It plays a pivotal role in the disease progression of cancer, mental retardation, atherosclerosis, and arthritis. The initial response of a cell to a migration-promoting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

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).

Cell Biolabs CytoSelect™ Cell Migration Assay Kit provides a robust system for the quantitative determination of cell migration. This Trial Size kit contains sufficient reagents for the evaluation of 4 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.

The CytoSelect™ Cell Migration Assay 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).

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-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-T): One 24-well plate containing 4 cell culture inserts (3 µm pore size)
2. Cell Detachment Solution (Part No. 10101-T): One 2 mL tube
3. 4X Lysis Buffer (Part No. 10102-T): One 2 mL tube
4. CyQuant® GR Dye (Part No. 10103-T): One 10 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 $CaCl_2$ 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.

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 x 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 µL of media containing 10% fetal bovine serum or desired chemoattractant(s) to the lower well of the migration plate.
4. Add 100 µL 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 µL of Cell Detachment Solution. Incubate 30 minutes at 37ºC.
   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 µL of the 500 µL medium solution containing migratory cells (step 5) to the well that contains 200 µL of Cell Detachment Solution for the same migration assay sample (step 7). Mix well, transfer 180 µL 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 µL dye to 370 µL of 4X Lysis Buffer).
10. Add 60 µL 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 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 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.

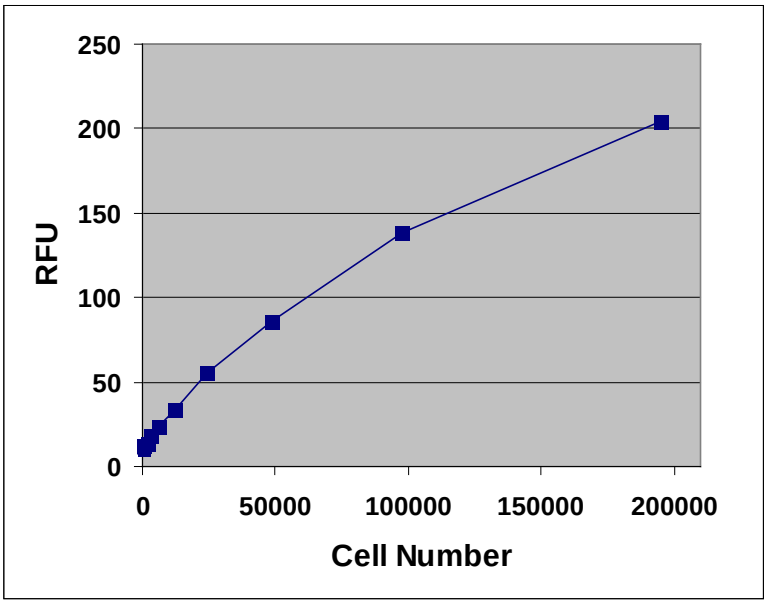


Figure 1: **Quantitation of Human promyelocytic cell line HL-60**. HL-60 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150 µL cell suspension was mixed with 50 µL of 4X Lysis Buffer/dye).

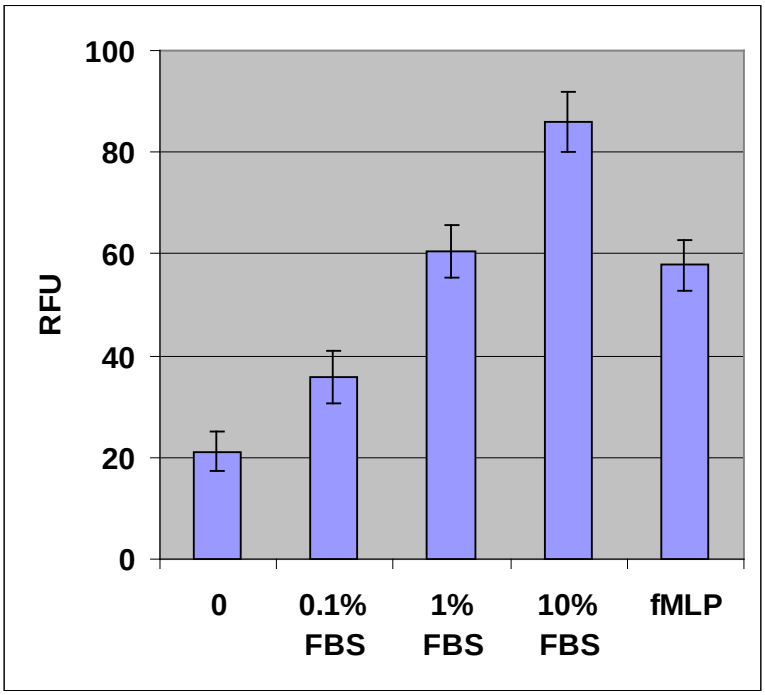


Figure 2. HL-60 Chemotaxis. 250,000 cells/well of HL-60 were allowed to migrate toward FBS or fMLP (100 nM) for 1 hr. Migratory cells were quantified by CyQuant® GR Dye as described in Assay Protocol.

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. Słoniecka, M. et al. (2015). Substance P enhances keratocyte migration and neutrophil recruitment through interleukin-8. *Mol Pharmacol.* doi:10.1124/mol.115.101014.
2. 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.
3. Roh, K. B. et al. (2014). Synephrine inhibits eotaxin-1 expression via the STAT6 signaling pathway. *Molecules.* **19**:11883-11895.
4. 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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