Product Manual

CytoSelect™ 24-Well Cell Haptotaxis Assay (8 µm, Collagen I-Coated, Fluorometric Format)

Catalog Number

CBA-101-COL **12 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

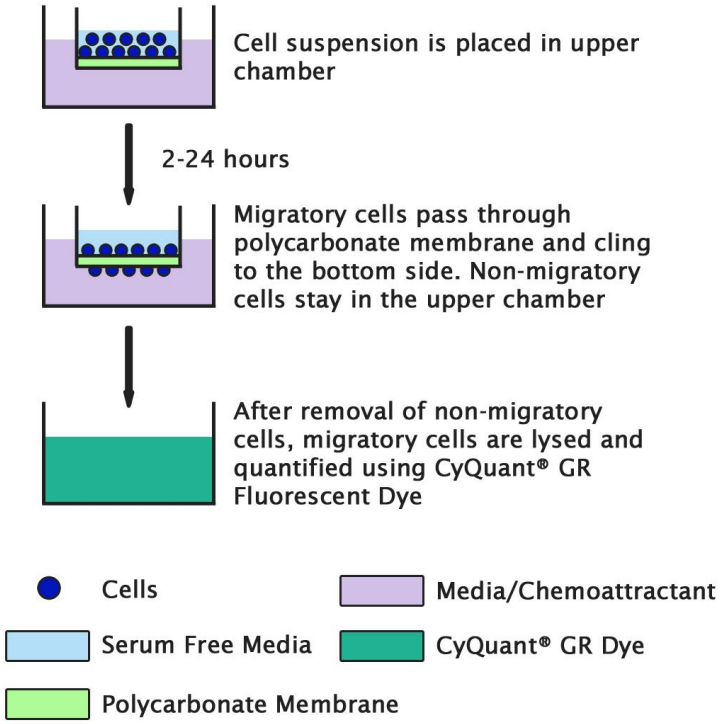
Introduction

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.

Cell Biolabs CytoSelect™ Cell Haptotaxis Assay Kit utilizes polycarbonate membrane inserts (8 µm pore size) to assay the migratory properties of cells, the bottom side of the insert is coated with Collagen I. The kit contains sufficient reagents for the evaluation of 12 samples. The 8 µm pore size is optimal for epithelial and fibroblast cell migration. The kit does not require you to prelabel the cells with Calcein AM. Migratory cells are lysed and detected by the patented CyQuant® GR Dye.

The CytoSelect™ Cell Haptotaxis 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 the gradient of extracellular matrix density (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 lysed and detected by the patented CyQuant® GR Dye.

Assay Principle



Related Products

1. CBA-101-C: CytoSelect™ 24-Well Cell Migration and Invasion Assay (8µm, Fluorometric)
2. CBA-101-FN: CytoSelect™ 24-Well Cell Haptotaxis Assay (Fibronectin, Fluorometric)
3. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)

4. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
5. CBA-111: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Fluorometric)

Kit Components

1. 24-well Migration Plate (Part No. 10001-COL): One 24-well plate containing 12 cell culture inserts (8 µm pore size, bottom side coated with collagen I)
2. 4X Lysis Buffer (Part No. 10102): One 5 mL bottle
3. CyQuant® GR Dye (Part No. 10103): One 25 µL tube
4. Cotton Swabs (Part No. 11004): 40 each
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 0.5-1.0 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. Use cotton-tipped swabs to gently remove non-migratory cells from the interior of the inserts. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter.

7. Prepare sufficient 1X Lysis Buffer/CyQuant® GR dye solution for all samples by diluting the dye 1:300 in 1X Lysis Buffer (for example, add 900 µL of H_2O to 300 µL of 4X Lysis Buffer, then add 4 µL dye to 1.2 mL of 1X Lysis Buffer).
8. Transfer the insert to a clean well containing 300 µL of 1X Lysis Buffer/CyQuant® GR dye solution and incubate for 10 minutes at room temperature.
9. Transfer 200 µL of the solution to 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 Haptotaxis Assay Kit. One should use the data below for reference only.

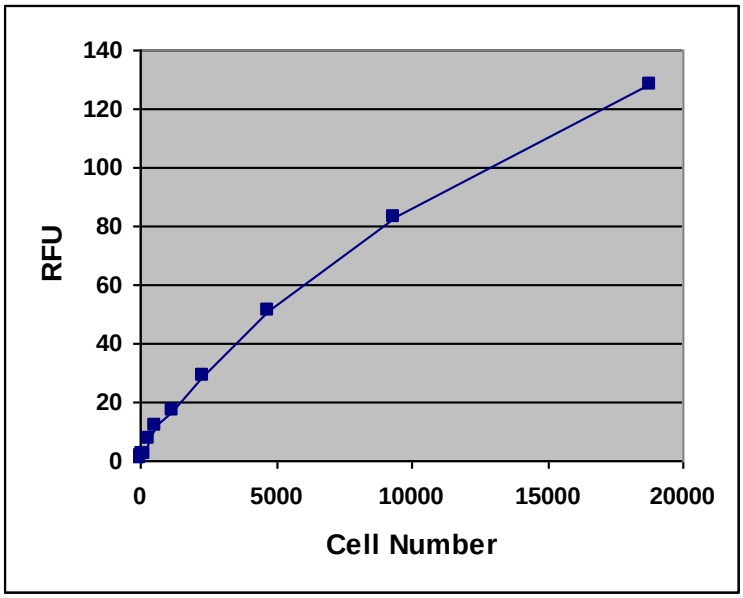
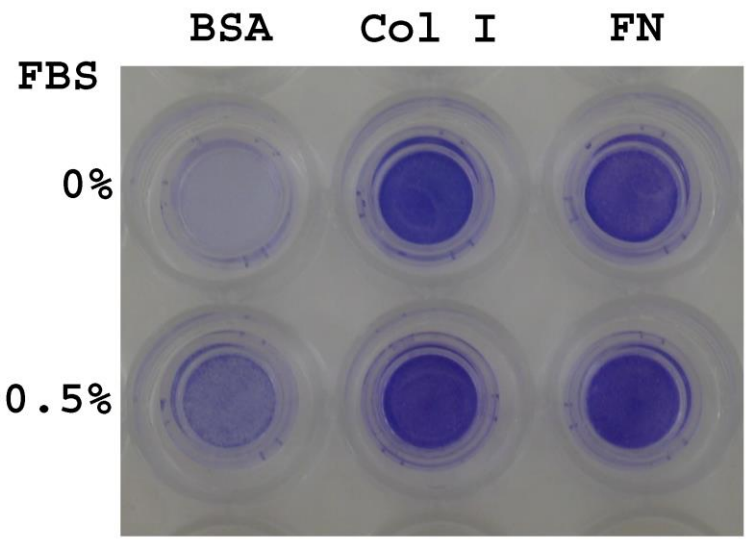


Figure 1: **Quantitation of MDA-231**. MDA-231 cells were titrated in culture medium, then subsequently lysed and detected with 1X Lysis Buffer/Cyquant® GR Dye.



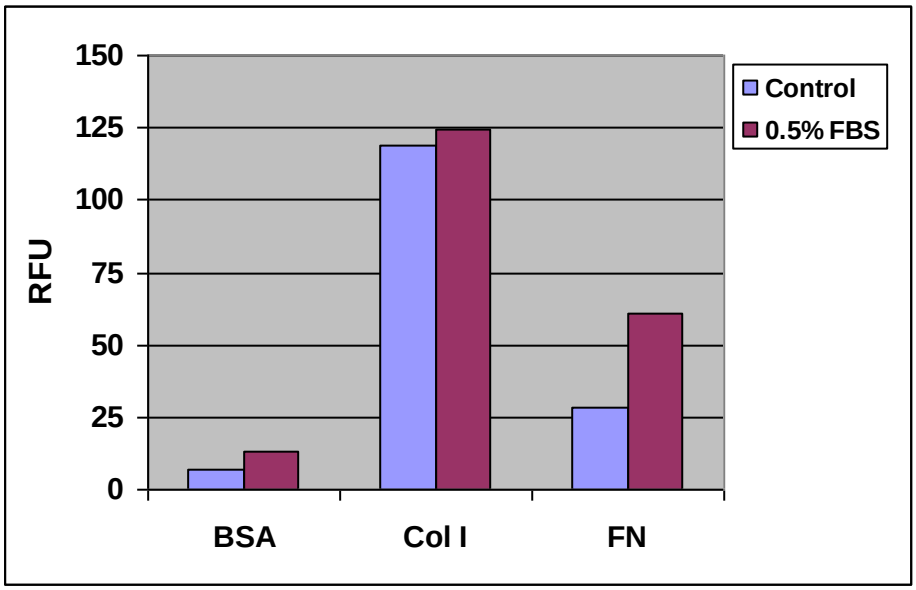


Figure 2. Breast Cancer MDA-231 Cell Haptotaxis and Chemotaxis. MDA-231 cells were seeded at 150,000 cells/well and allowed to migrate toward FBS for 4 hrs. Migratory cells on the bottom of the polycarbonate membrane were stained (top panel picture) and quantified by CyQuant® GR Dye as described in Assay Protocol (bottom panel figure).

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. Bezhaeva, T. et al. (2018). Relaxin receptor deficiency promotes vascular inflammation and impairs outward remodeling in arteriovenous fistulas. *FASEB J*. fj201800437R. doi: 10.1096/fj.201800437R.
2. Singh, D.R. et al. (2016). The SAM domain inhibits EphA2 interactions in the plasma membrane. *Biochim Biophys. Acta* doi: 10.1016/j.bbamcr.2016.10.011.
3. Singh, D. R. et al. (2015). EphA2 unliganded dimers suppress EphA2 pro-tumorigenic signaling. *J Biol Chem.* doi:10.1074/jbc.M115.676866.

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Contact Information

Cell Biolabs, Inc.
5628 Copley Drive
San Diego, CA 92111
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com