Product Manual

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

Catalog Number

CBA-100-COL **12 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

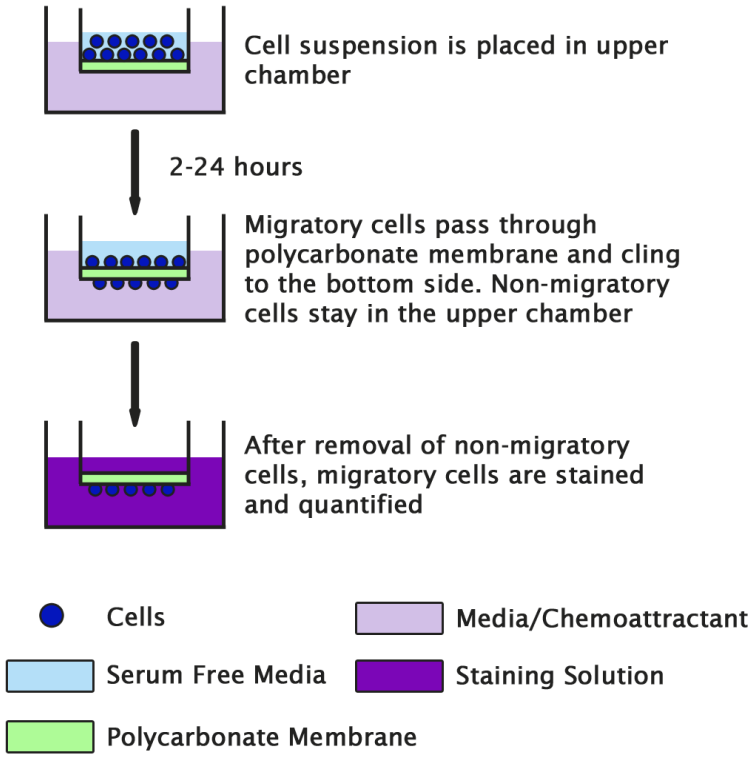
Introduction

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. 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 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. However, in the case of leukocyte chemotaxis, a smaller pore size (3 µm) is recommended.

Assay Principle

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 stained and quantified.



Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-100-FN: CytoSelect™ 24-Well Cell Haptotaxis Assay (Fibronectin, Colorimetric)
3. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
4. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
5. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)

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. Cell Stain Solution (Part No. 11002): One 10 mL bottle
3. Extraction Solution (Part No. 11003): One 10 mL bottle
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 microtiter plate
7. Microtiter 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. Wet the ends of 2-3 cotton-tipped swabs with water, flatten the ends of the swabs by pressing them against a clean hard surface, and gently

swab the interior of the inserts to remove non-migratory cells. Take care not to puncture the polycarbonate membrane. Be sure to remove cells on the inside perimeter of the insert.

7. Transfer the insert to a clean well containing 400 µL of Cell Stain Solution and incubate for 10 minutes at room temperature.
8. Gently wash the stained inserts several times in a beaker of water. Allow the inserts to air dry.
9. (optional) Count migratory cells with a light microscope under high magnification objective, with at least three individual fields per insert.
10. Transfer each insert to an empty well, adding 200 µL of Extraction Solution per well, then incubating 10 minutes on an orbital shaker.
11. Transfer 100 µL from each sample to a 96-well microtiter plate and measure the OD 560nm in a plate reader.

Example of Results

The following figures demonstrate typical with the CytoSelect™ Cell Haptotaxis Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

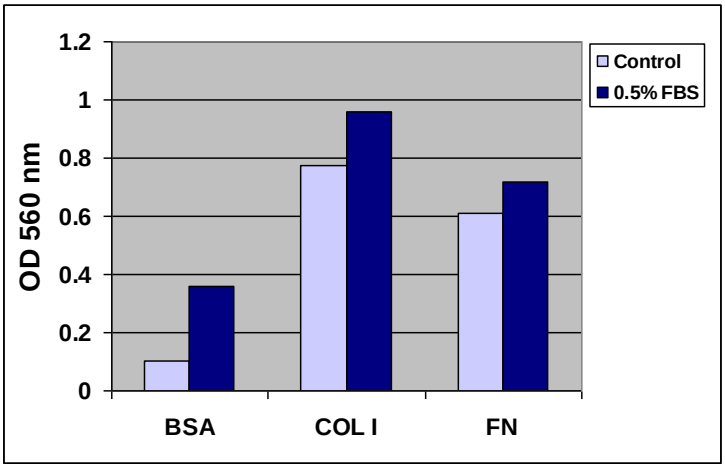


Figure 1. Breast Cancer MDA-231 Cell Haptotaxis and Chemotaxis. MDA-231 cells were seeded at 150,000 cells/well and allowed to migrate toward FBS for 4 hrs. Migratory cells on the bottom of the polycarbonate membrane were quantified at OD 560nm after extraction.

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.
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Recent Product Citations

1. Orlandella, F.M. et al. (2019). miR-650 promotes motility of anaplastic thyroid cancer cells by targeting PPP2CA. *Endocrine*. doi: 10.1007/s12020-019-01910-3.
2. Xu, Y. et al. (2018). Sipa1 deficiency unleashes a host-immune mechanism eradicating chronic myelogenous leukemia-initiating cells. *Nat Commun*. **9**(1):914. doi: 10.1038/s41467-018-03307-8.

3. Yamauchi, A., et al. (2017). Evaluation of pancreatic cancer cell migration with multiple parameters in vitro by using an optical real-time cell mobility assay device. *BMC Cancer*. **17**(1):234. doi: 10.1186/s12885-017-3218-4.
4. Herrera, I. et al. (2013). Matrix metalloproteinase (MMP)-1 induces lung alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption. *J. Biol. Chem*. **288**:25964-25975.
5. Niccoli, S. et al. (2012).The Asian-American E6 variant protein of human papillomavirus 16 alone is sufficient to promote immortalization, transformation, and migration of primary human foreskin keratinocytes. *J Virol*. **86**:12384-12396.
6. Kamiya, K. et al. (2007). Protein Kinase C delta activated adhesion regulates vascular smooth muscle cell migration. *J. Surg. Res*. **141**:91-96.

Warranty

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