Datasheet for ABIN2344836

CytoSelect™ 24-well Cell Haptotaxis Assay (8 µm), COL-coated, Colorimetric

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Overview

Quantity:	12 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Sample Type:	Serum, Cell Samples
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Characteristics:	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.
Components:	1. 24-well Migration Plate : One 24-well plate containing 12 cell culture inserts (8 µm pore size, bottom side coated with collagen I) 2. Cell Stain Solution : One 10 mL bottle 3 3. Extraction Solution : One 10 mL bottle 4. Cotton Swabs : 40 each 5. Forceps: One each
Material not included:	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

Product Details

4. Cell culture incubator (37 °C, 5 % CO2 atmosphere)
5. Light microscope
6. 96-well microtiter plate
7. Microtiter plate reader

Target Details

Background:	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).

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Fully quantify haptotaxis with no manual cell counting • Measure haptotaxis in less than 6 hours with most cell types • Membrane inserts are precoated on the bottom with Collagen
Plate:	Pre-coated
Protocol:	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.
Assay Procedure:	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 106 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.

Application Details

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. 4

Restrictions:	For Research Use only

Handling

Storage:	4 °C
Storage Comment:	Store all components at 4°C.

Publications

Product cited in:

Zheng, Cheng, Fu, Fan, Wang, Yu, Sun, Tian, Wei: "Targeting LUNX Inhibits Non-Small Cell Lung Cancer Growth and Metastasis." in: **Cancer research**, (2015) (PubMed).

Herrera, Cisneros, Maldonado, Ramírez, Ortiz-Quintero, Anso, Chandel, Selman, Pardo: "Matrix metalloproteinase (MMP)-1 induces lung alveolar epithelial cell migration and proliferation, protects from apoptosis, and represses mitochondrial oxygen consumption." in: **The Journal of biological chemistry**, Vol. 288, Issue 36, pp. 25964-75, (2013) (PubMed).

Niccoli, Abraham, Richard, Zehbe: "The Asian-American E6 variant protein of human papillomavirus 16 alone is sufficient to promote immortalization, transformation, and migration of primary human foreskin keratinocytes." in: **Journal of virology**, Vol. 86, Issue 22, pp. 12384-96, (2012) (PubMed).

Kamiya, Ryer, Sakakibara, Zohlman, Kent, Liu: "Protein kinase C delta activated adhesion regulates vascular smooth muscle cell migration." in: **The Journal of surgical research**, Vol.

141, Issue 1, pp. 91-6, (2007) (PubMed).

Images

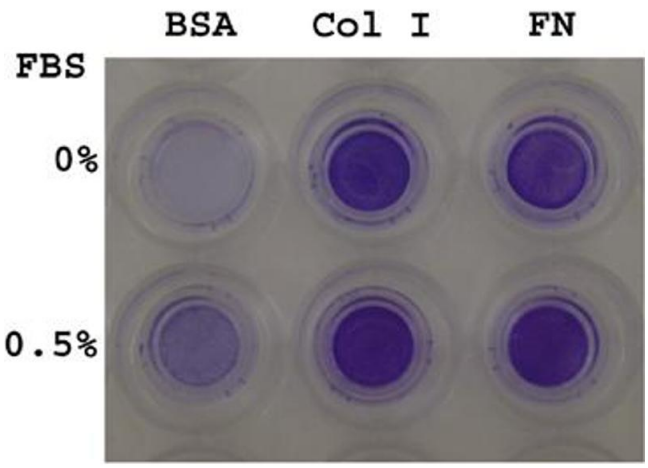

Image 1. CytoSelect™ 24-Well Cell Haptotaxis Assay.MDA-231 cells were seeded at 150,000 cells/well and allowed to migrate toward FBS for 4 hours. Migratory cells, on the bottom of the migration membrane, were stained according to the assay protocol.