Datasheet for ABIN2344838

CytoSelect™ 24-Well Cell Migration Assay (8 µm, Fluorometric Format)

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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:	Fluorometric
Characteristics:	CytoSelect™ Cell Migration Assay Kit utilizes polycarbonate membrane inserts (8 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). 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 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. The CytoSelect™ Cell Migration 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 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).

Product Details

Components:	1. 24-well Migration Plate : One 24-well plate containing 4 cell culture inserts (8 µm pore size) 2. Cell Detachment Solution : One 2 mL tube 3. 4X Lysis Buffer : One 2 mL tube 4. CyQuant® GR Dye : One 10 µL tube 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 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

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 chemotaxis with no manual cell counting • Measure chemotaxis in less than 6 hours with most cell types • Membrane inserts are uncoated to allow use with any chemoattractant
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. Transfer the insert to a clean well containing 225 µL of Cell Detachment Solution. Incubate 30 minutes at 37 °C.
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. 4
8. 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).
9. Add 75 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well containing cells and 225 µL of Cell Detachment Solution. Incubate 20 minutes at room temperature.
10. Transfer 200 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read fluorescence with a fluorescence plate reader at 480 nm/520 nm.

Restrictions:	For Research Use only

Handling

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

Publications

Product cited in:

S?oniecka, Le Roux, Zhou, Danielson: "Substance P Enhances Keratocyte Migration and Neutrophil Recruitment through Interleukin-8." in: **Molecular pharmacology**, Vol. 89, Issue 2, pp. 215-25, (2016) (PubMed).

Ibrahim, Kulshrestha, Katara, Amin, Beaman: "Cancer derived peptide of vacuolar ATPase 'a2' isoform promotes neutrophil migration by autocrine secretion of IL-8." in: **Scientific reports**, Vol. 6, pp. 36865, (2016) (PubMed).

Banerjee, Hernandez, Garcia, Kangsamaksin, Sbiroli, Andrews, Forrester, Wei, Kadenhe-Chiweshe, Shawber, Kitajewski, Kandel, Yamashiro: "Notch suppresses angiogenesis and progression of hepatic metastases." in: **Cancer research**, Vol. 75, Issue 8, pp. 1592-602, (2015) (PubMed).

Izhak, Wildbaum, Weinberg, Uri, Shaked, Alami, Dumont, Friedman, Stein, Karin: "Predominant expression of CCL2 at the tumor site of prostate cancer patients directs a selective loss of immunological tolerance to CCL2 that could be amplified in a beneficial manner." in: **Journal of immunology (Baltimore, Md. : 1950)**, Vol. 184, Issue 2, pp. 1092-101, (2010) (PubMed).

Izhak, Wildbaum, Zohar, Anunu, Klapper, Elkeles, Seagal, Yefenof, Ayalon-Soffer, Karin: "A novel

recombinant fusion protein encoding a 20-amino acid residue of the third extracellular (E3) domain of CCR2 neutralizes the biological activity of CCL2." in: **Journal of immunology (Baltimore, Md. : 1950)**, Vol. 183, Issue 1, pp. 732-9, (2009) (PubMed).

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