Datasheet for ABIN2344852

CytoSelect™ 96-well Cell Migration Assay (5 µm), Fluorometric

19 Publications

Go to Product page

Overview

Quantity:	96 tests
Reactivity:	Mammalian
Application:	Cellular Assay (CA)

Product Details

Brand:	CytoSelect™
Sample Type:	Serum, Cell Samples
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	CytoSelect™ 96-well Cell Migration Assay Kit utilizes a polycarbonate membrane plate (5 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 with CyQuant® GR Dye. CytoSelect™ 96-well Cell Migration Assay Kit provides a robust system for the quantitative determination of cell migration. The kit contains sufficient reagents for the evaluation of 96 samples. The 5 µm pore size is optimal for monocyte and macrophage cell migration. However, in the case of leukocyte chemotaxis, a smaller pore size (3 µm) is recommended. The CytoSelect™ Cell Migration Assay Kit contains a polycarbonate membrane chamber (5 µm pore size) in a 96-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 with CyQuant® GR Dye.

Product Details

Components:	1. 96-well Cell Migration Plate : One sterile 96-well plate (see Figure 1 for components) 2. 96-well Cell Harvesting Tray : One 96-well tray 3. Cell Detachment Solution : One 20 mL bottle 4. 4X Lysis Buffer : One 10 mL bottle 5. CyQuant® GR Dye : One 75 µL tube
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. FBS or desired chemoattractant 5. Cell culture incubator (37 °C, 5 % CO2 atmosphere) 6. Light microscope 7. 96-well plate suitable for a fluorescence plate reader 8. Fluorescence plate reader 4 Top Plate Cover Middle Migration Plate Membrane Chamber Bottom Feeder Tray : Components of the 96-well Cell Migration Plate.

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.

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. Allow the 96-well Migration Plate to warm up at room temperature for 10 minutes. 2. Prepare a cell suspension containing 0.5-5.0 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. Under sterile conditions, separate the cover and membrane chamber from the 96-well Migration Plate. 4. Add 150 µL of media containing 10 % fetal bovine serum or desired chemoattractant(s) to

Application Details

the wells of the feeder tray. 5

5. Place the membrane chamber back into the feeder tray (containing chemoattractant solution). Ensure no bubbles are trapped under the membrane.
6. Gently mix the cell suspension (without chemoattractant) from step 2 and add 100 µL to the membrane chamber.
7. Finally, cover the plate and transfer to a cell culture incubator for 2-24 hours.
8. Just prior to the end of the incubation, pipette 150 µL of prewarmed Cell Detachment Solution into wells of the clean, 96-Well Cell Harvesting Tray (provided).
9. Carefully remove the 96-well Migration Plate from the incubator. Separate the membrane chamber from the feeder tray. Note: Retain the feeder tray for step
10. 10. Remove the cells/media from the top side of the membrane chamber by aspirating or inverting. Place the membrane chamber into the Cell Harvesting Tray containing 150 µL of Cell Detachment Solution (step 8). Incubate 30 minutes at 37 °C.
11. Completely dislodge the cells from the underside of the membrane by gently tilting the membrane chamber several times in the Cell Detachment Solution.
12. In a clean 96-well plate (not provided), combine 75 µL of media from the feeder tray (step 9) with 75 µL of the detachment solution (step 11).
13. 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).
14. Add 50 µL of 4X Lysis Buffer/CyQuant® GR dye solution to each well (already containing 150 µL of Cell Detachment Solution). Incubate 20 minutes at room temperature.
15. Transfer 150 µL of the mixture to a 96-well plate suitable for fluorescence measurement. Read the 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:

Vazquez Rodriguez, Abrahamsson, Jensen, Dabrosin: "Estradiol Promotes Breast Cancer Cell Migration via Recruitment and Activation of Neutrophils." in: **Cancer immunology research**, Vol. 5, Issue 3, pp. 234-247, (2017) (PubMed).

Shikhagaie, Björklund, Mjösberg, Erjefält, Cornelissen, Ros, Bal, Koning, Mebius, Mori, Bruchard, Blom, Spits: "Neuropilin-1 Is Expressed on Lymphoid Tissue Residing LTi-like Group 3 Innate Lymphoid Cells and Associated with Ectopic Lymphoid Aggregates." in: **Cell reports**, Vol. 18, Issue 7, pp. 1761-1773, (2017) (PubMed).

Yao, Abe, Kawasaki, Akbar, Matsuura, Onji, Hiasa: "Characterization of Liver Monocytic Myeloid-Derived Suppressor Cells and Their Role in a Murine Model of Non-Alcoholic Fatty Liver Disease." in: **PLoS ONE**, Vol. 11, Issue 2, pp. e0149948, (2016) (PubMed).

Cao, Cui, Wu, Zha, Wang, Parks, Yu, Shi, Xue: "Myeloid Deletion of α1AMPK Exacerbates Atherosclerosis in LDL Receptor Knockout (LDLRKO) Mice." in: **Diabetes**, Vol. 65, Issue 6, pp. 1565-76, (2016) (PubMed).

Luo, Wei, Chen, Zhao, Huang, Chen: "Methylation-mediated loss of SFRP2 enhances melanoma cell invasion via Wnt signaling." in: **American journal of translational research**, Vol. 8, Issue 3, pp. 1502-9, (2016) (PubMed).

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