Datasheet for ABIN2344842

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

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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 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.
Components:	1. 24-well Migration Plate : One 24-well plate containing 12 cell culture inserts (8 µm pore size,

Product Details

	bottom side coated with collagen I) 2. 4X Lysis Buffer : One 5 mL bottle 3. CyQuant® GR Dye : One 25 µL tube 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 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 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
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.

Application Details

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 H2O to 300 µL of 4X Lysis Buffer, then add 4 µL dye to 1.2 mL of 1X Lysis Buffer). 4
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.

Restrictions:	For Research Use only

Handling

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

Publications

Product cited in:

Singh, Ahmed, Paul, Gedam, Pasquale, Hristova: "The SAM domain inhibits EphA2 interactions in the plasma membrane." in: **Biochimica et biophysica acta**, Vol. 1864, Issue 1, pp. 31-38, (2016) (PubMed).

Singh, Ahmed, King, Gupta, Salotto, Pasquale, Hristova: "EphA2 Receptor Unliganded Dimers Suppress EphA2 Pro-tumorigenic Signaling." in: **The Journal of biological chemistry**, Vol. 290, Issue 45, pp. 27271-9, (2015) (PubMed).