Product Manual

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

Catalog Number

CBA-101     12 assays
CBA-101-5   5 x 12 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures
**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 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).

Cell Biolabs CytoSelect™ Cell Migration Assay Kit provides a robust system for the quantitative determination of cell migration. 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.

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).
Assay Principle

Related Products
1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-101-COL: CytoSelect™ 24-Well Cell Haptotaxis Assay (Collagen I, Fluorometric)
3. CBA-101-FN: CytoSelect™ 24-Well Cell Haptotaxis Assay (Fibronectin, Fluorometric)
4. CBA-102: CytoSelect™ 24-Well Cell Migration Assay (5µm, Fluorometric)
5. CBA-103: CytoSelect™ 24-Well Cell Migration Assay (3µm, Fluorometric)
6. CBA-104: CytoSelect™ 96-Well Cell Migration Assay (3 µm, Fluorometric)
7. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8µm, Fluorometric)
8. CBA-110: CytoSelect™ 24-Well Cell Invasion Assay (Basement Membrane, Colorimetric)
9. CBA-125: Radius™ 24-Well Cell Migration Assay (Microscopy)
10. CBA-126: Radius™ 96-Well Cell Migration Assay (Microscopy)
11. CBA-130: CytoSelect™ 96-Well Cell Transformation Assay (Soft Agar Colony Formation)

Kit Components
1. 24-well Migration Plate (Part No. 10001): One 24-well plate containing 12 cell culture inserts (8 µm pore size)
2. Cell Detachment Solution (Part No. 10101): One 5 mL bottle
3. **4X Lysis Buffer** (Part No. 10102): One 5 mL bottle
4. **CyQuant® GR Dye** (Part No. 10103): One 25 µL tube
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 (37°C, 5% CO\(_2\) atmosphere)
5. Light microscope
6. 96-well plate suitable for a fluorescence plate reader
7. Fluorescence plate reader

**Storage**
Store all components at 4°C.

**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. 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.
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.
Example of Results
The following figures demonstrate typical with the CytoSelect™ Cell Migration Assay Kit. Fluorescence measurement was performed on SpectraMax Gemini XS Fluorometer (Molecular Devices) with a 485/538 nm filter set and 530 nm cutoff. One should use the data below for reference only. This data should not be used to interpret actual results.

Figure 1: Quantitation of Human HT-1080. HT-1080 cells were titrated in Cell Detachment Buffer, then subsequently lysed and detected with 4X Lysis Buffer/Cyquant® GR Dye (150 µL cell suspension was mixed with 50 µL of 4X Lysis Buffer/dye).
Figure 2. Human Fibrosarcoma HT-1080 Cell Migration. HT-1080 was seeded at 30,000 cells/well and allowed to migrate toward FBS for 4 hrs in the presence or absence of 2 µM Cytochalasin D. Migratory cells on the bottom of the polycarbonate membrane were stained (top panel picture) and quantified by CyQuant® GR Dye as described in the Assay Protocol.

References

Recent Product Citations

License Information
This product is provided under an intellectual property license from Life Technologies Corporation. The purchase of this product conveys to the buyer the non-transferable right to use the purchased
product and components of the product only in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The sale of this product is expressly conditioned on the buyer not using the product or its components, or any materials made using the product or its components, in any activity to generate revenue, which may include, but is not limited to use of the product or its components: (i) in manufacturing; (ii) to provide a service, information, or data in return for payment; (iii) for therapeutic, diagnostic or prophylactic purposes; or (iv) for resale, regardless of whether they are resold for use in research. For information on purchasing a license to this product for purposes other than as described above, contact Life Technologies Corporation, 5791 Van Allen Way, Carlsbad CA 92008 USA or outlicensing@lifetech.com.

**Warranty**

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS’ sole obligation and purchaser’s exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

**Contact Information**

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com

©2004-2023: Cell Biolabs, Inc. - All rights reserved. No part of these works may be reproduced in any form without permissions in writing.