Product Manual

CytoSelect™ Tumor Transendothelial Migration Assay

Catalog Number

CBA-216	24 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

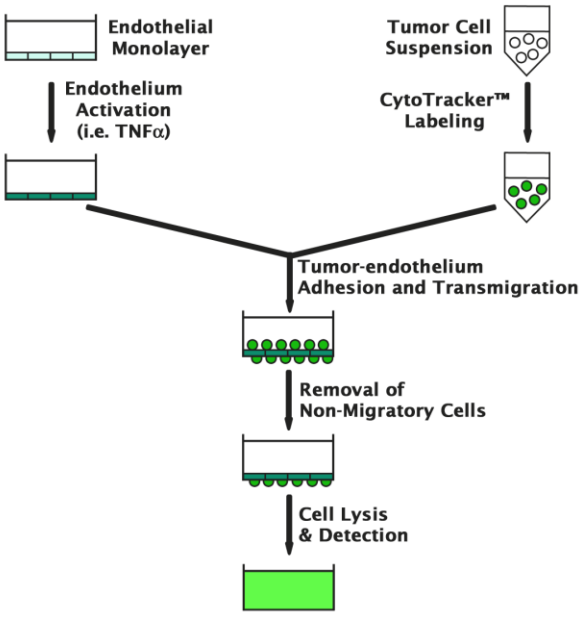
CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Cancer metastasis comprises several steps. First tumor cells are shed into the blood stream (intravasation), circulating in the blood, and finally transmigrating out of the vessels (extravasation) into a new location in the body.

The initial arrest and attachment of tumor cells to vascular endothelium precedes their extravasation from the blood stream and is a crucial step in the tumor metastatic cascade. Tumor cell extravasation is equivalent, in many respects, to the entry of leukocytes into inflammatory tissue. Leukocyte extravasation consists of multiple, consecutive processes including the capture of circulating leukocytes, subsequent leukocyte rolling, arrest, firm adhesion and transmigration. Increasing evidence suggests that tumor cell adhesion to the endothelial lining and transendothelial migration is influenced by endothelial activation or tissue-specific differences in endothelium and depends on the expression of specific cell surface molecules. E-Selectin and Vascular Cell Adhesion Molecule-1 (VCAM-1) appear to play a pivotal role in the tumor-EC interaction.

Cell Biolabs' CytoSelect™ Tumor Transendothelial Migration Assay provides a robust system for the quantitative determination of tumor-endothelium interactions and transmigrations. The kit contains sufficient reagents for the evaluation of 24 assays in a 24-well plate.



Related Products

1. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8µm, Colorimetric)
2. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8µm, Fluorometric)
3. CBA-105: CytoSelect™ 96-Well Cell Migration Assay (5µm, Fluorometric)
4. CBA-106: CytoSelect™ 96-Well Cell Migration Assay (8 µm, Fluorometric)
5. CBA-212: CytoSelect™ Leukocyte Transmigration Assay

Kit Components

1. 24-well Migration Plate (Part No. 121601): One 24-well plate containing 24 cell culture inserts (8 µm pore size)
2. 500X CytoTracker™ Solution (Part No. 12151): One 100 µL tube
3. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
4. TNFα (Part No. 12105): One 100 µL tube of 10 µg/mL TNFα in sterile 1X PBS/0.1%BSA
5. Cotton Swabs (Part No. 11004): 40 each
6. Forceps (Part No. 11005): One each

Materials Not Supplied

1. Endothelial cells and cell culture medium
2. 24-well plate
3. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
4. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
5. 1X PBS containing 2 mM $CaCl_2$ and 2 mM $MgCl_2$
6. Light microscope
7. 96-well plate suitable for a fluorescence plate reader
8. Fluorescence plate reader

Storage

CytoTracker™ Solution and TNFa should be removed from the kit and stored at -20ºC immediately. Store all other components at 4ºC.

Preparation of Reagents

- 1X Lysis Buffer: Prepare a 1X Lysis Buffer by diluting the provided 4X stock 1:4 in deionized water. Store the diluted solution at room temperature.

Assay Protocol

1. Add 50,000-100,000 endothelial cells in 300 µL medium to each insert in a 24-well plate containing 500 µL of culture medium.
2. Culture cells for 48-72 until the endothelial cells form a monolayer.
3. Treat endothelial cell monolayer with desired activator or inhibitor, such as TNFα.
4. Harvest cancer cells and prepare a cell suspension at 0.5 - 1.0 x 10^6 cells/ml in serum free media.
5. Add CytoTracker™ to a final concentration of 1X (for example, add 2 µL of 500X CytoTracker™ solution to 1.0 mL of cancer cell suspension). Incubate for 60 min at 37ºC in a cell culture incubator. Spin down cells at 1000 rpm for 2 minutes, aspirate the medium and wash cell pellet with serum free media. Repeat the wash twice. Resuspend the cell pellet at 0.25 - 1.0 x 10^6

cells/ml in serum free media. Agents that inhibit or stimulate cell migration may be added directly to the cell suspension.

6. Carefully remove endothelial culture medium from migration insert without disturbing the endothelial monolayer and transfer the insert to another well containing 500 µL of tumor cell culture media including 10% fetal bovine serum or desired chemoattractant(s).
7. Add 300 µL of the cell suspension solution to the inside of each insert.
8. Incubate for 2-24 hours in a cell culture incubator.
9. 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.
10. Transfer the insert to a clean well containing 200 µL of 1X Lysis Buffer. Incubate 5 minutes at room temperature with shaking.
11. Transfer 100 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 Cell Biolabs CytoSelect™ Tumor Transendothelial Migration Assay Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

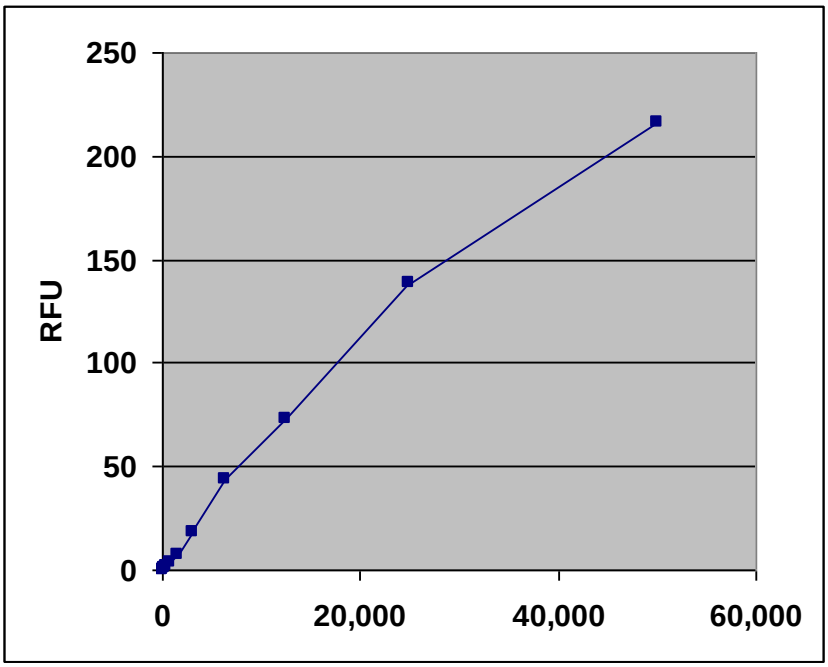


Figure 1. Quantitation of Human Breast Cancer MDA-231 Cells. CytoTracker™ labeled MDA-231 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 µL of cell suspension was mixed with 75 µL of 2X Lysis Buffer). Fluorescence was quantified as described in the Assay Protocol.

References

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Recent Product Citations

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