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Product Manual

# CytoSelect™ Tumor Transendothelial Migration Assay

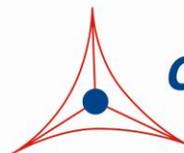
Catalog Number

CBA-216

24 assays

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

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*Creating Solutions for Life Science Research*

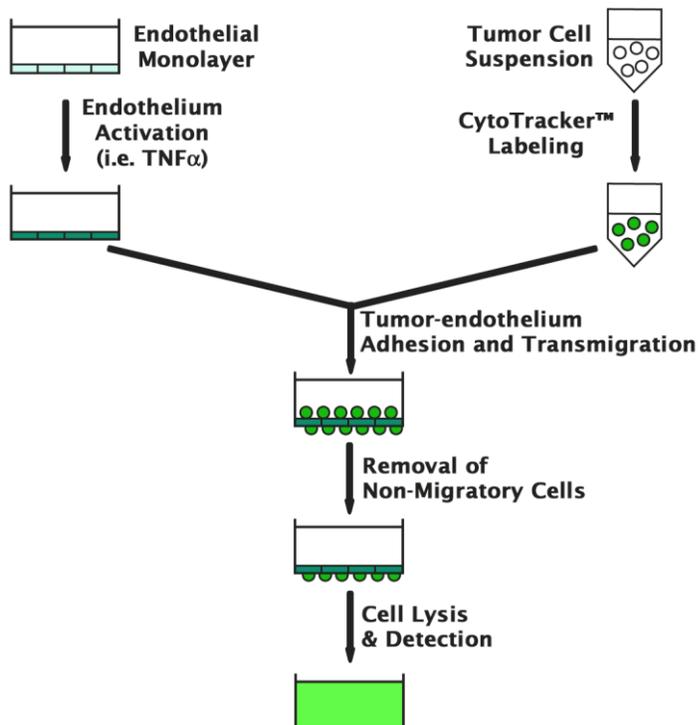
## **Introduction**

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.

## **Assay Principle**



## **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-120: CytoSelect™ 24-Well Wound Healing Assay
6. CBA-125: Radius™ 24-Well Cell Migration Assay
7. CBA-126: Radius™ 96-Well Cell Migration Assay
8. CBA-210: CytoSelect™ Leukocyte-Endothelium Adhesion Assay
9. CBA-211: CytoSelect™ Leukocyte-Epithelium Adhesion Assay
10. CBA-212: CytoSelect™ Leukocyte Transmigration Assay
11. CBA-215: CytoSelect™ Tumor-Endothelium Adhesion 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<sub>2</sub> and 2 mM MgCl<sub>2</sub>
4. Cell culture incubator (37°C, 5% CO<sub>2</sub> atmosphere)
5. 1X PBS containing 2 mM CaCl<sub>2</sub> and 2 mM MgCl<sub>2</sub>
6. Light microscope
7. 96-well plate suitable for a fluorescence plate reader
8. Fluorescence plate reader

## **Storage**

CytoTracker™ Solution and TNF $\alpha$  should be removed from the kit and stored at -20°C immediately. Store all other components at 4°C.

## **Preparation of Reagents**

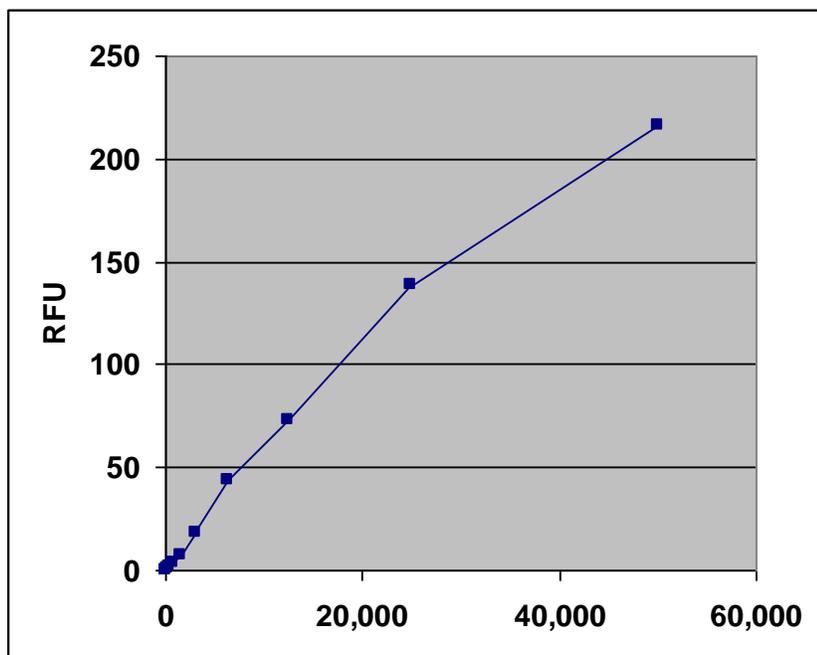
- 1X Lysis Buffer: Prepare a 1X Lysis Buffer by diluting the provided 4X stock 1:4 in deionized water. Store the diluted solution at room temperature.

## **Assay Protocol**

1. Add 50,000-100,000 endothelial cells in 300  $\mu$ L medium to each insert in a 24-well plate containing 500  $\mu$ L of culture medium.
2. Culture cells for 48-72 until the endothelial cells form a monolayer.
3. Treat endothelial cell monolayer or cancer cells with desired activator or inhibitor, such as TNF $\alpha$ .
4. Harvest cancer cells and prepare a cell suspension at 0.5 - 1.0 x 10<sup>6</sup> cells/ml in serum free media. Agents that inhibit or stimulate cell migration can be added directly to the cell suspension.
5. Add CytoTracker™ to a final concentration of 1X (for example, add 2  $\mu$ L of 500X CytoTracker™ solution to 1.0 mL of cancer cell suspension). Incubate for 60 min at 37°C 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<sup>6</sup> cells/ml in serum free media.
6. Carefully remove endothelial culture medium from migration insert without disturbing the endothelial monolayer and transfer the insert to another well containing 500  $\mu$ L of tumor cell culture media including 10% fetal bovine serum or desired chemoattractant(s).
7. Add 300  $\mu$ L 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  $\mu$ L of 1X Lysis Buffer. Incubate 5 minutes at room temperature with shaking.
11. Transfer 100  $\mu$ L 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. 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 Breast Cancer MDA-231 Cells.** CytoTracker™ labeled MDA-231 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75  $\mu$ L of cell suspension was mixed with 75  $\mu$ L of 2X Lysis Buffer). Fluorescence was quantified as described in the Assay Protocol.

## References

1. Zen K. and Parkos C. A. (2003) *Curr Opin Cell Biol.* **15**, 557-64.

## Recent Product Citations

1. Park, G. B. et al. (2015). Regulation of ADAM10 and ADAM17 by sorafenib inhibits epithelial-to-mesenchymal transition in Epstein-Barr virus–infected retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci.* **56**:5162-5173.
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4. Haidari, M. et al. (2014). Disruption of endothelial adherens junctions by high glucose is mediated by protein kinase C- $\beta$ -dependent vascular endothelial cadherin tyrosine phosphorylation. *Cardiovasc Diabetol.* **13**:112.

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7. Yang, H. and H.E. Grossniklaus (2010). Constitutive overexpression of pigment epithelium derived factor inhibition of ocular melanoma growth and metastasis. *Invest. Ophthalmol. Vis. Sci.* **51**:28-34.
8. Liu, K. et al. (2007). Lentivirus mediated gene transfer of PEDF results in decreased uveal melanoma transendothelial migration. *Invest. Ophthalmol. Vis. Sci.* **48**:5244.

### **Warranty**

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