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

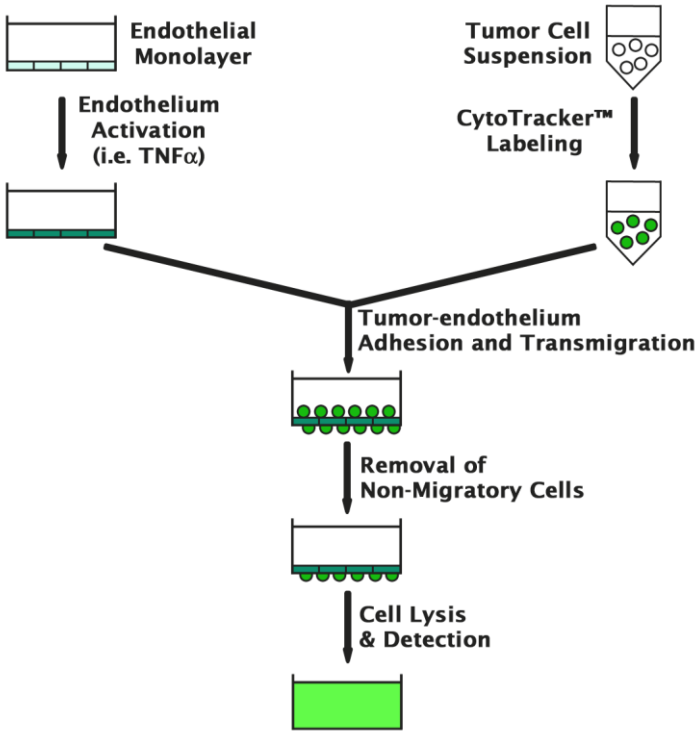
Introduction

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.

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 $CaCl_2$ 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. 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.

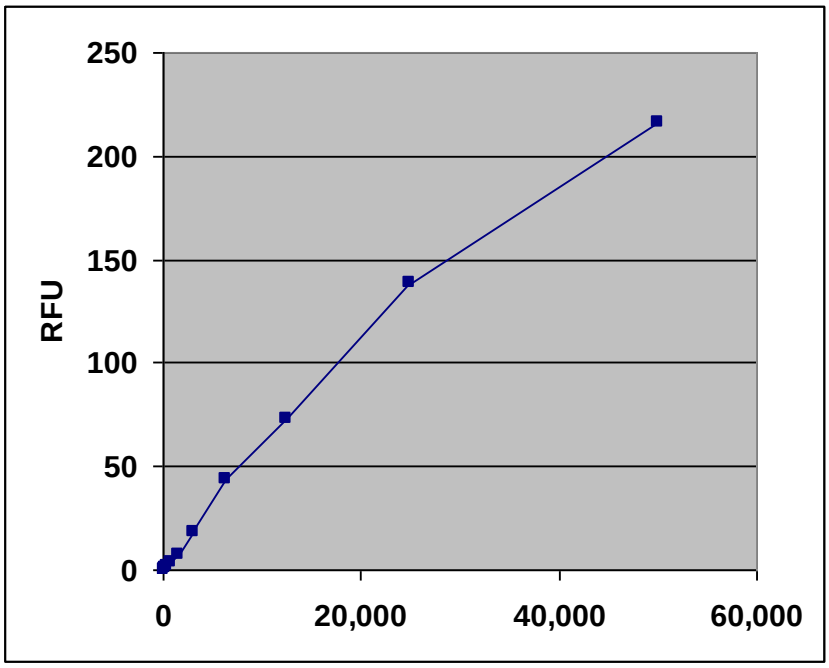


Figure 1. Quantitation of Human Breast Cancer MDA-231 Cells. CytoTracker™ labeled MDA-231 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 µL of cell suspension was mixed with 75 µL of 2X Lysis Buffer). Fluorescence was quantified as described in the Assay Protocol.

References

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

Recent Product Citations

1. Huang, M.B. et al. (2022). Novel secretion modification region (SMR) peptide exhibits anti-metastatic properties in human breast cancer cells. *Sci Rep*. **12**(1):13204. doi: 10.1038/s41598-022-17534-z.
2. Park, G.B. et al. (2021). TrkB/C-induced HOXC6 activation enhances the ADAM8-mediated metastasis of chemoresistant colon cancer cells. *Mol Med Rep*. **23**(6):423. doi: 10.3892/mmr.2021.12062.
3. Sasahira, T. et al. (2021). SERPINE2 is an oral cancer-promoting factor that induces angiogenesis and lymphangiogenesis. *Int J Clin Oncol*. doi: 10.1007/s10147-021-01970-4.
4. Sasahira, T. et al. (2021). Identification of oral squamous cell carcinoma markers MUC2 and SPRR1B downstream of TANGO. *J Cancer Res Clin Oncol*. doi: 10.1007/s00432-021-03568-9.

5. Kong, J. et al. (2020). ICAM-1 Activates Platelets and Promotes Endothelial Permeability through VE- Cadherin after Insufficient Radiofrequency Ablation. *Adv. Sci*. doi: 10.1002/advs.202002228.
6. Park, G.B. et al. (2020). GLUT5 regulation by AKT1/3-miR-125b-5p downregulation induces migratory activity and drug resistance in TLR-modified colorectal cancer cells. *Carcinogenesis*. doi: 10.1093/carcin/bgaa074.
7. Shimomura, H. et al. (2019). Non-SMC Condensin I Complex Subunit H (NCAPH) Is Associated with Lymphangiogenesis and Drug Resistance in Oral Squamous Cell Carcinoma. *J Clin Med*. **9**(1). pii: E72. doi: 10.3390/jcm9010072.
8. Park, G.B. et al. (2019). Modified TLR-mediated downregulation of miR-125b-5p enhances CD248 (endosialin)-induced metastasis and drug resistance in colorectal cancer cells. *Mol Carcinog*. doi: 10.1002/mc.23137.
9. Wang, S.Y. et al. (2019). High Expression of MicroRNA-196a is Associated with Progression of Hepatocellular Carcinoma in Younger Patients. *Cancers (Basel)*. **11**(10). pii: E1549. doi: 10.3390/cancers11101549.
10. Park, G.B. et al. (2019). MicroRNA-503-5p Inhibits the CD97-Mediated JAK2/STAT3 Pathway in Metastatic or Paclitaxel-Resistant Ovarian Cancer Cells. *Neoplasia*. **21**(2):206-215. doi: 10.1016/j.neo.2018.12.005.
11. Fukushima, R. et al. (2018). Overexpression of Translocation Associated Membrane Protein 2 Leading to Cancer-Associated Matrix Metalloproteinase Activation as a Putative Metastatic Factor for Human Oral Cancer. *J Cancer*. **9**(18):3326-3333. doi: 10.7150/jca.25666.
12. Toeda, Y. et al. (2018). FBLIM1 enhances oral cancer malignancy via modulation of the epidermal growth factor receptor pathway. *Mol Carcinog*. **57**(12):1690-1697. doi: 10.1002/mc.22889.
13. Sasahira, T. et al. (2018). NIPA-like domain containing 1 is a novel tumor-promoting factor in oral squamous cell carcinoma. *J Cancer Res Clin Oncol*. **144**(5):875-882. doi: 10.1007/s00432-018-2612-x.
14. Park, G.B., and Kim, D. (2017). Insulin-like growth factor-1 activates different catalytic subunits p110 of PI3K in a cell-type-dependent manner to induce lipogenesis-dependent epithelial-mesenchymal transition through the regulation of ADAM10 and ADAM17. *Mol Cell Biochem*. doi: 10.1007/s11010-017-3148-0.
15. Choong L.Y., et al. (2017). Lee WH, et al. (2017). TRPV4 plays a role in breast cancer cell migration via Ca^{2+}-dependent activation of AKT and downregulation of E-cadherin cell cortex protein. *Oncogenesis* **6** (5):e338. doi: 10.1038/oncsis.2017.39.
16. Park, G.B. and Kim, D. (2017). PI3K Catalytic Isoform Alteration Promotes the LIMK1-related Metastasis Through the PAK1 or ROCK1/2 Activation in Cigarette Smoke-exposed Ovarian Cancer Cells. *Anticancer Res*. **37** (4):1805-1818.
17. Fife, C.M. et al. (2016). Stathmin mediates neuroblastoma metastasis in a tubulin-independent manner via RhoA/ROCK signaling and enhanced transendothelial migration. *Oncogene*. doi:10.1038/onc.2016.220.
18. Waghray, M. et al. (2016). GM-CSF mediates mesenchymal-epithelial crosstalk in pancreatic cancer. *Cancer Discov*. Doi:10.1158/2159-8290.CD-15-0947.
19. 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.
20. Park, G. B. et al. (2015). Silencing of galectin-3 represses osteosarcoma cell migration and invasion through inhibition of FAK/Src/Lyn activation and β-catenin expression and increases susceptibility to chemotherapeutic agents. *Int J Oncol.* **46**:185-194.

21. Choi, S. H. et al. (2014). MMP9 processing of HSPB1 regulates tumor progression. *PLoS One.* **9**:e85509.
22. Haidari, M. et al. (2014). Disruption of endothelial adherens junctions by high glucose is mediated by protein kinase C-β-dependent vascular endothelial cadherin tyrosine phosphorylation. *Cardiovasc Diabetol*. **13**:112.
23. Park, G.B. et al. (2014). The Epstein-Barr virus causes epithelial-mesenchymal transition in human corneal epithelial cells via Syk/Src and Akt/Erk signaling pathways. *Invest. Ophthalmol. Vis. Sci.* **55**:1770-1779.
24. Xu, Z. et al. (2010). Role of pancreatic stellate cells in pancreatic cancer metastasis. *Am. J. Pathol.*, **177**:2585-2596.
25. Yang, H. and H.E. Grossniklaus (2010). Consitutive overexpression of pigment epithelium derived factor inhibition of ocular melanoma growth and metastasis. *Invest. Ophthalmol. Vis. Sci.* **51**:28-34.
26. Liu, K. et al. (2007). Lentivirus mediated gene transfer of PEDF results in decreased uveal melanoma transendothelial migration. Invest. *Opthalmol. Vis. Sci.* **48**:5244.

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