Datasheet for ABIN2344906

CytoSelect™ Tumor Transendothelial Migration Assay

8 Publications

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Overview

Quantity:	24 tests
Application:	Biochemical Assay (BCA)

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples, Serum
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	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.
Components:	1. 24-well Migration Plate : One 24-well plate containing 24 cell culture inserts (8 µm pore size) 2. 500X CytoTracker™ Solution : One 100 µL tube 3. 4X Lysis Buffer : One 10 mL bottle 4. TNFα : One 100 µL tube of 10 µg/mL TNFα in sterile 1X PBS/0.1%BSA 5. Cotton Swabs : 40 each 6. Forceps : One each
Material not included:	1. Endothelial cells and cell culture medium 2. 24-well plate 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. 1X PBS containing 2 mM CaCl2 and 2 mM MgCl2 6. Light microscope 7. 96-well plate suitable for a fluorescence plate reader

Product Details

8. Fluorescence plate reader 3

Target Details

Background:	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.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quantify interactions between endothelium and tumor cells • Fully quantify cell transmigration with no manual cell counting • Highly sensitive results on a fluorescence plate reader
Reagent Preparation:	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 Procedure:	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 or cancer cells with desired activator or inhibitor, such as TNFα. 6 4. Harvest cancer cells and prepare a cell suspension at 0.5 - 1.0 x 10 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 µL 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 6 with serum free media. Repeat the wash twice. Resuspend the cell pellet at

Application Details

0.25 - 1.0 x 10 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 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. 4

Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C
Storage Comment:	CytoTracker™ Solution and TNFa should be removed from the kit and stored at -20°C immediately. Store all other components at 4°C.

Publications

Product cited in:	Waghray, Yalamanchili, Dziubinski, Zeinali, Erkkinen, Yang, Schradle, Urs, Pasca Di Magliano, Welling, Palmbos, Abel, Sahai, Nagrath, Wang, Simeone: "GM-CSF Mediates Mesenchymal-Epithelial Cross-talk in Pancreatic Cancer." in: **Cancer discovery**, Vol. 6, Issue 8, pp. 886-99, (2016) (PubMed). Fife, Sagnella, Teo, Pouha, Byrne, Yeap, Ng, Davis, McCarroll, Kavallaris: "Stathmin mediates neuroblastoma metastasis in a tubulin-independent manner via RhoA/ROCK signaling and enhanced transendothelial migration." in: **Oncogene**, Vol. 36, Issue 4, pp. 501-511, (2016) (PubMed). Park, Kim, Kim, Kim, Sun, Roh, Yang, Hur: "Regulation of ADAM10 and ADAM17 by Sorafenib Inhibits Epithelial-to-Mesenchymal Transition in Epstein-Barr Virus-Infected Retinal Pigment Epithelial Cells." in: **Investigative ophthalmology & visual science**, Vol. 56, Issue 9, pp. 5162-73, (2015) (PubMed).

Park, Kim, Kim, Lee, Kim, Hur et al.: "Silencing of galectin-3 represses osteosarcoma cell migration and invasion through inhibition of FAK/Src/Lyn activation and ?-catenin expression and increases susceptibility to chemotherapeutic ..." in: **International journal of oncology**, Vol. 46, Issue 1, pp. 185-94, (2014) (PubMed).

Park, Kim, Kim, Kim, Lee, Yang, Hur: "The Epstein-Barr virus causes epithelial-mesenchymal transition in human corneal epithelial cells via Syk/src and Akt/Erk signaling pathways." in: **Investigative ophthalmology & visual science**, Vol. 55, Issue 3, pp. 1770-9, (2014) (PubMed).

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