Datasheet for ABIN3198656

CytoSelect™ 96-well Leukocyte-endothelium Adhesion Kit

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Overview

Quantity:	100 tests
Application:	Biochemical Assay (BCA)

Product Details

Brand:	CytoSelect™
Sample Type:	Cell Samples, Serum
Analytical Method:	Quantitative
Detection Method:	Fluorometric
Characteristics:	CytoSelect™ Leukocyte-endothelium Adhesion Assay provides a robust system for the quantitative determination of leukocyte-endothelium interactions. The kit contains sufficient reagents for the evaluation of 100 assays in a 96-well plate.
Components:	1. 500X LeukoTracker™ Solution : One 100 µL tube 2. Gelatin Solution : One 12 mL bottle of sterile 0.1 % Gelatin in 1X PBS 3. 4X Lysis Buffer : One 10 mL bottle 3 4. 10X Wash Buffer : One 20 mL bottle 5. TNFα : One 100 µL tube of 10 µg/mL TNFα in sterile 1X PBS/0.1%BSA
Material not included:	1. Endothelial cells and cell culture medium 2. 96-well or 48-well plate 3. Serum free medium, such as DMEM containing 0.5 % BSA, 2 mM CaCl2 and 2 mM MgCl2 4. Sterile 1X PBS 5. Cell culture incubator (37 °C, 5 % CO2 atmosphere) 6. Light microscope 7. 96-well plate suitable for a fluorescence plate reader 8. Fluorescence plate reader

Target Details

Background:	Leukocyte extravasation into perivascular tissue plays a key role in inflammatory diseases. This recruitment requires leukocyte interaction with vascular endothelium and consists of multiple, consecutive processes including the capture of circulating leukocytes, subsequent leukocyte rolling, arrest, firm adhesion and transmigration (Figure 1). This multistep paradigm is realized by sequential activation-dependent interactions between endothelial cell adhesion molecules and their specific ligands on leukocytes. The first step of transient adhesion and rolling is known to be mediated by an interaction of leukocyte or endothelial cell selectins and their oligosaccharide-bearing ligands. Arrest and firm adhesion of leukocytes to endothelium is dependent on the activation of β2 integrins like Mac-1 or LFA-1 on the leukocyte cell surface, followed by interaction with endothelial cell proteins belonging to the Ig superfamily such as ICAM-1.

Application Details

Application Notes:	Optimal working dilution should be determined by the investigator.
Comment:	• Quantify interactions between leukocytes and the endothelium • Fully quantitative with no manual cell counting • Highly sensitive results on a fluorescence plate reader
Reagent Preparation:	1X Wash Buffer: Prepare a 1X Wash Buffer by diluting the provided 10X stock 1:10 in deionized water. Store the diluted solution at room temperature. 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. Gelatin Coating 1. Under sterile conditions, add 200 µL of the Gelatin Solution to each well of a 48-well tissue culture treated plate, or 100 µL of the Gelatin Solution to each well of a 96-well tissue culture treated plate. 2. Incubate for 60 min at 37 °C in a cell culture incubator. 3. Wash twice with sterile 1X PBS. Aspirate the final wash before use.
Assay Procedure:	1. Add 50,000-100,000 endothelial cells/well to the Gelatin-coated 48-well or 96-well plate. 2. Culture cells for 48-72 until the endothelial cells form a monolayer. 3. Treat endothelial cell monolayer or leukocyte with desired activator or inhibitor for 6-12 hrs. 4 6 4. Harvest leukocytes and prepare a cell suspension at 1.0 x 10 cells/mL in serum free media. Add LeukoTracker to a final concentration of 1X (for example, add 2 µL of 500X LeukoTracker™ solution to 1.0 mL of leukocyte cell suspension). 5. 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. 6 Resuspend the cell pellet at 0.25 - 1.0 x 10 cells/mL in serum free media. 6. Aspirate endothelial culture media and wash once with serum free media. Add 200 µL of the cell suspension to each well already containing the endothelial monolayer.

Application Details

7. Incubate for 30-90 min in a cell culture incubator.
8. Carefully discard or aspirate the media from each well (Note: Do not allow wells to dry). Gently wash each well 3 times with 250 µL 1X Wash Buffer.
9. (Optional) Count the adherent leukocytes under an inverted fluorescence microscope, average at least three separate fields per well.
10. Aspirate the final wash and add 150 µL of 1X Lysis Buffer to each well containing cells. 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.

Restrictions:	For Research Use only

Handling

Storage:	4 °C/-20 °C
Storage Comment:	LeukoTracker™ 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:

Rossignoli, Shang, Gladh, Moessinger, Foroughi Asl, Talukdar, Franzén, Mueller, Björkegren, Folestad, Skogsberg: "Poliovirus Receptor-Related 2: A Cholesterol-Responsive Gene Affecting Atherosclerosis Development by Modulating Leukocyte Migration." in: **Arteriosclerosis, thrombosis, and vascular biology**, Vol. 37, Issue 3, pp. 534-542, (2017) (PubMed).

Huang, Qiu, Zeng, Xiao, Shi, Zou, Ye, Liang, Yang, Xu: "Niclosamide inhibits the inflammatory and angiogenic activation of human umbilical vein endothelial cells." in: **Inflammation research : official journal of the European Histamine Research Society ... [et al.]**, Vol. 64, Issue 12, pp. 1023-32, (2016) (PubMed).

He, Chen, Martin, Zhang, Sangwung, Woo, Tremoulet, Shimizu, Jain, Burns, Shyy: "miR-483 Targeting of CTGF Suppresses Endothelial-to-Mesenchymal Transition: Therapeutic Implications in Kawasaki Disease." in: **Circulation research**, Vol. 120, Issue 2, pp. 354-365, (2016) (PubMed).

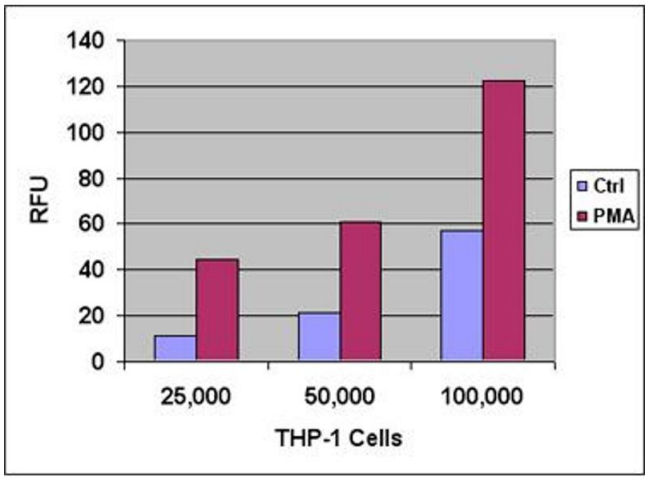
Cao, Cui, Wu, Zha, Wang, Parks, Yu, Shi, Xue: "Myeloid Deletion of α1AMPK Exacerbates Atherosclerosis in LDL Receptor Knockout (LDLRKO) Mice." in: **Diabetes**, Vol. 65, Issue 6, pp. 1565-76, (2016) (PubMed).

Publications

Ibrahim, Elshafey, Sellak, Hussein, El-Sherbiny, Abdelsaid, Rizk, Beasley, Tawfik, Smith, Al-Shabrawey: "A lipidomic screen of hyperglycemia-treated HRECs links 12/15-Lipoxygenase to microvascular dysfunction during diabetic retinopathy via NADPH oxidase." in: **Journal of lipid research**, Vol. 56, Issue 3, pp. 599-611, (2015) (PubMed).

There are more publications referencing this product on: Product page

Images



Cellular Assay

Image 1. Human Monocytic THP-1 Adhesion to HUVEC Monolayer. HUVEC monolayer in a 48-well plate was treated with 1 μM PMA for 12 hours. LeukoTracker™ labeled THP-1 cells were allowed to attach to HUVEC monolayer for 1 hour. Adherent cells were lysed and quantified as described in the assay protocol.