Product Manual

CytoSelect™ Leukocyte-Endothelium Adhesion Assay

Catalog Number

CBA-210 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

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.

Cell Biolabs' 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.

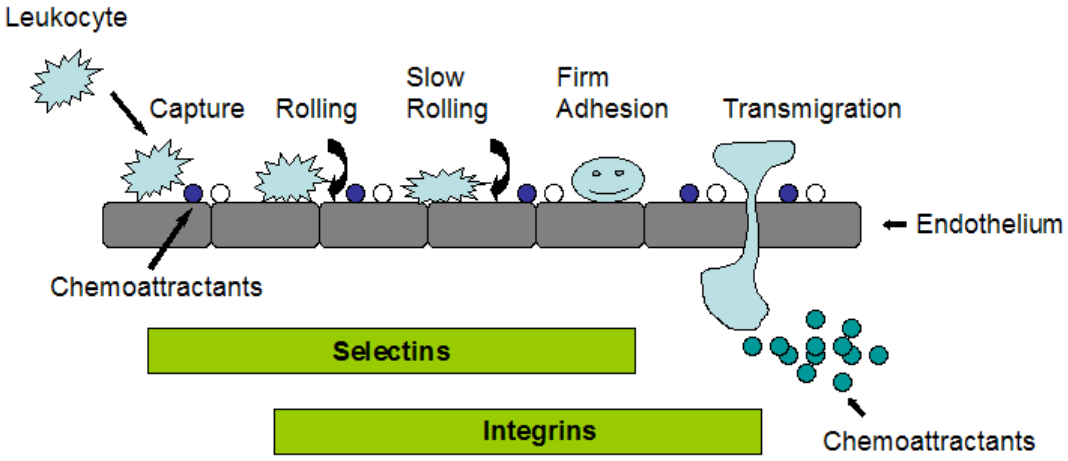
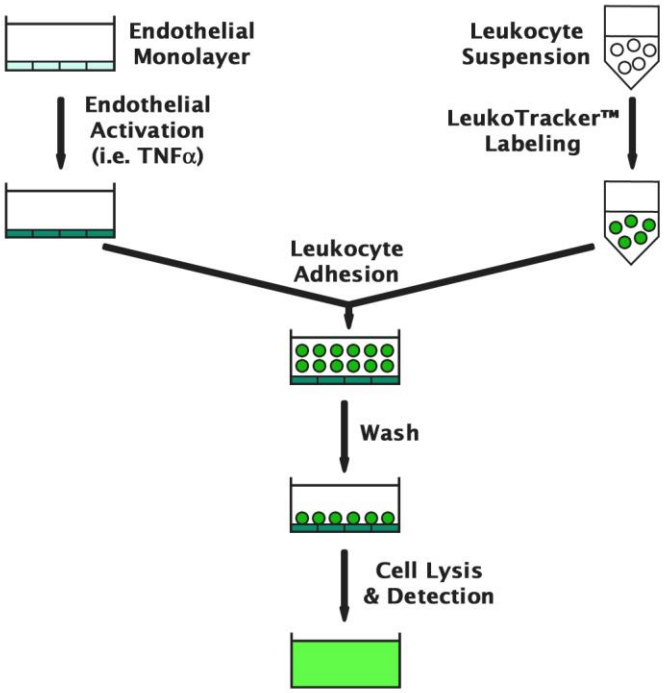


Figure 1. The Leukocyte-Endothelium Adhesion Cascade.



Related Products

1. CBA-052: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen I, Colorimetric)
2. CBA-053: CytoSelect™ 48-Well Cell Adhesion Assay (Collagen I, Fluorometric)
3. CBA-070: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Colorimetric)
4. CBA-071: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Fluorometric)
5. CBA-200: Endothelial Tube Formation Assay (In Vitro Angiogenesis)
6. CBA-212: CytoSelect™ Leukocyte Transmigration Assay
7. CBA-216: CytoSelect™ Tumor Transendothelial Migration Assay
8. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Kit Components

1. 500X LeukoTracker™ Solution (Part No. 12101): One 100 µL tube
2. Gelatin Solution (Part No. 12102): One 12 mL bottle of sterile 0.1 % Gelatin in 1X PBS
3. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
4. 10X Wash Buffer (Part No. 12104): One 20 mL bottle
5. TNFα (Part No. 12105): One 100 µL tube of 10 µg/mL TNFα in sterile 1X PBS/0.1%BSA

Materials Not Supplied

1. Endothelial cells and cell culture medium
2. Leukocytes
3. 96-well or 48-well tissue culture plate
4. Serum free medium, such as DMEM containing 0.5% BSA, 2 mM $CaCl_2$ and 2 mM $MgCl_2$
5. Sterile 1X PBS
6. Cell culture incubator (37ºC, 5% CO_2 atmosphere)
7. Light microscope
8. 96-well plate suitable for a fluorescence plate reader
9. Fluorescence plate reader

Storage

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

Preparation of Reagents

- 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 37ºC in a cell culture incubator.
3. Wash twice with sterile 1X PBS. Aspirate the final wash before use.

Assay Protocol

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. Harvest leukocytes and prepare a cell suspension at 1.0 x 10^6 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 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.
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.
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.

Example of Results

The following figures demonstrate typical with Cell Biolabs CytoSelect™ Leukocyte-endothelium Adhesion 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.

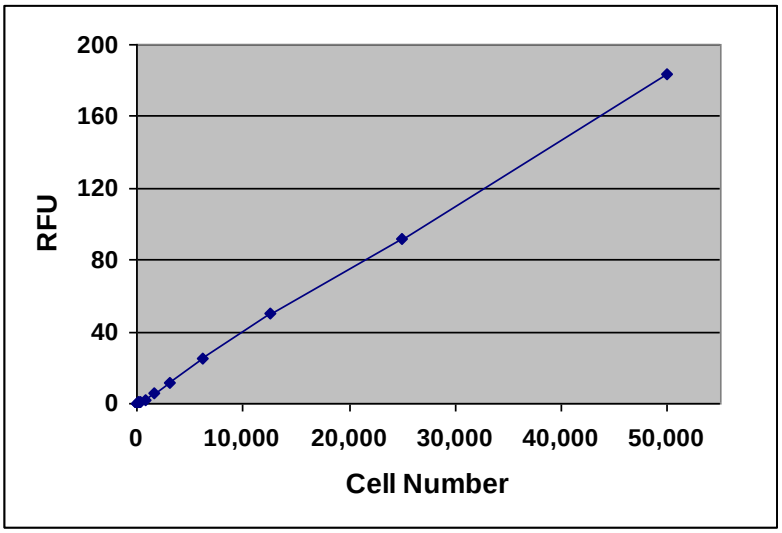


Figure 2. Quantitation of Human Monocytic THP-1. LeukoTracker™ labeled THP-1 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 µL of cell suspension was mixed with 75 µL of 2X Lysis Buffer). Fluorescence was quantified as described in Assay Protocol.

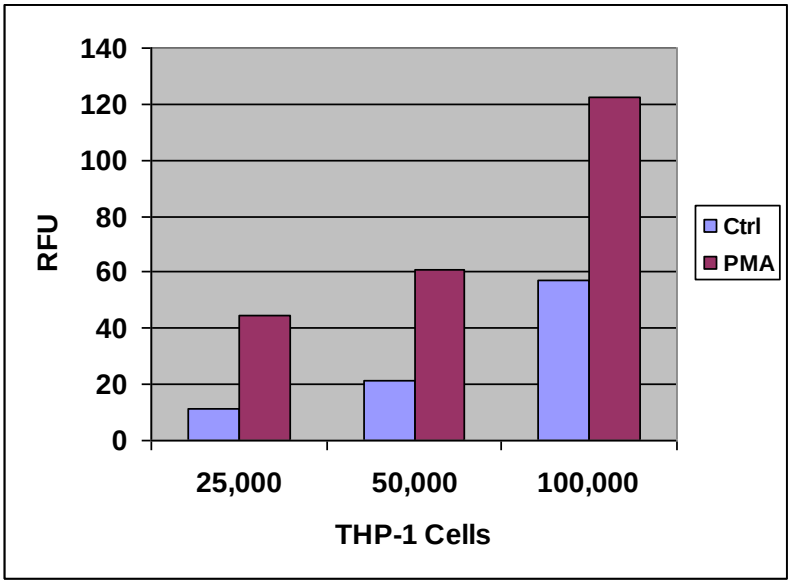


Figure 3. Human Monocytic THP-1 Adhesion to HUVEC Monolayer. HUVEC monolayer in 48-well plate was treated with 1 µM PMA for 12 hrs. LeukoTracker™ labeled THP-1 cells were allowed to attach to HUVEC monolayer for 1 hr. Adherent cells were lysed and quantified by as described in the Assay Protocol.

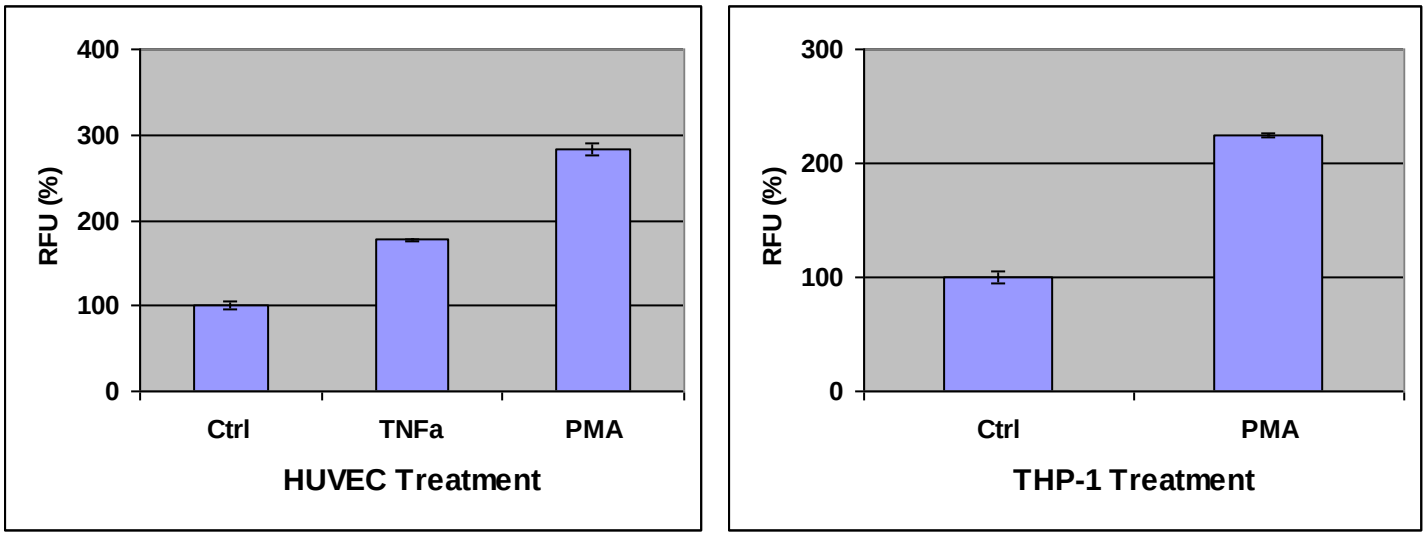


Figure 4. Cytokine Activation of Human Monocytic THP-1 Adhesion to HUVEC. HUVEC monolayer (left) or THP-1 cells (right) were treated with 50 ng/mL TNFα or 1 µM PMA for 12 hrs. LeukoTracker™ labeled THP-1 cells (50,000 cells/well) were allowed to attach to HUVEC monolayer for 1 hr. Adherent cells were lysed and quantified by as described in the Assay Protocol.

References

1. Carlos T. M and Harlan J. M. (1994) *Blood* **84**, 2068-101.
2. Meldrum, D. R. (1998) *Am. J. Physiol.* **274**, R577-R595.

Recent Product Citations

1. Azúa-López, Z.R. et al. (2023). HDL anti-inflammatory function is impaired and associated with high SAA1 and low APOA4 levels in aneurysmal subarachnoid hemorrhage. *J Cereb Blood Flow Metab*. doi: 10.1177/0271678X231184806.
2. Revilla-González, G. et al. (2023). Changes in Adhesion and the Expression of Adhesion Molecules in PBMCs after Aneurysmal Subarachnoid Hemorrhage: Relation to Cerebral Vasospasm. *Transl Stroke Res*. doi: 10.1007/s12975-023-01136-6.
3. Zhao, Z. et al. (2021). Gout-induced endothelial impairment: The role of SREBP2 transactivation of YAP. *FASEB J*. **35**(6):e21613. doi: 10.1096/fj.202100337R.
4. Otto, M. et al. (2021). Apremilast effectively inhibits TNFα-induced vascular inflammation in human endothelial cells. *J Eur Acad Dermatol Venereol*. doi: 10.1111/jdv.17769.
5. Bravo, P.L.W. et a. (2021). Antithrombotic Effect of the Ethanol Extract of Angelica gigas Nakai (AGE 232). *Life (Basel)*. **11**(9):939. doi: 10.3390/life11090939.
6. Poursaleh, A. et al. (2021). Adhesion of monocytes and endothelial cells isolated from the human aorta suppresses by miRNA-PEI particles. *BMC Cardiovasc Disord*. **21**(1):395. doi: 10.1186/s12872-021-02203-2.
7. Gao, X. et al. (2021). Atherogenic oxoaldehyde of cholesterol induces innate immune response in monocytes and macrophages. *Cell Biochem Biophys*. doi: 10.1007/s12013-021-01004-w.
8. Johnson, M.D. et al. (2020). CC16 Binding to α4β1 Integrin (VLA-4) Protects Against Mycoplasma pneumoniae Infection. *Am J Respir Crit Care Med*. doi: 10.1164/rccm.202006-2576OC.
9. Guo, J. et al. (2020). Hsa-miR-23a-3p promotes atherogenesis in a novel mouse model of atherosclerosis. *J Lipid Res*. doi: 10.1194/jlr.RA120001121.
10. Nair, M. et al. (2020). Enhancing Antitumor Efficacy of Heavily Vascularized Tumors by RAMBO Virus through Decreased Tumor Endothelial Cell Activation. *Cancers (Basel)*. **12**(4). pii: E1040. doi: 10.3390/cancers12041040.
11. Sasaki, N. et al. (2020). Rapamycin promotes endothelial-mesenchymal transition during stress-induced premature senescence through the activation of autophagy. *Cell Commun Signal*. **18**(1):43. doi: 10.1186/s12964-020-00533-w.
12. Liang, J. et al. (2020). Attenuation of pristimerin on TNF-α-induced endothelial inflammation. *Int Immunopharmacol*. **82**:106326. doi: 10.1016/j.intimp.2020.106326.
13. Abu El-Asrar, A.M. et al. (2019). Galectin-1 studies in proliferative diabetic retinopathy. *Acta Ophthalmol*. doi: 10.1111/aos.14191.
14. Li, R. et al. (2018). Attenuation of antimalarial agent hydroxychloroquine on TNF-α-induced endothelial inflammation. *Int Immunopharmacol*. **63**:261-269. doi: 10.1016/j.intimp.2018.08.008.
15. Hong, B. et al. (2018). Suppression of HMGB1 Released in the Glioblastoma Tumor Microenvironment Reduces Tumoral Edema. *Mol Ther Oncolytics*. **12**:93-102. doi: 10.1016/j.omto.2018.11.005.
16. Huang, W. et al. (2018). Oridonin inhibits vascular inflammation by blocking NF-κB and MAPK activation. *Eur J Pharmacol*. **826**:133-139. doi: 10.1016/j.ejphar.2018.02.044.
17. Ramella, M. et al. (2017). Endothelial MMP-9 drives the inflammatory response in abdominal aortic aneurysm (AAA). *Am J Transl Res*. **9**(12):5485-5495.
18. Wojkowska, D.J. et al. (2017). Interleukin 17A Promotes Lymphocytes Adhesion and Induces CCL2 and CXCL1 Release from Brain Endothelial Cells. *Int. J. Mol. Sci.* doi:10.3390/ijms18051000.

19. He, M. et al. (2016). miR-483 targeting of CTGF suppresses endothelial-to-mesenchymal transition: therapeutic implications in Kawasaki Disease. *Circ. Res*. **120**(2):354-365. doi:10.1161/CIRCRESAHA.116.310233.
20. Rossignoli A, et al. (2017). Poliovirus Receptor-Related 2: A Cholesterol-Responsive Gene Affecting Atherosclerosis Development by Modulating Leukocyte Migration. *Arterioscler Thromb Vasc Biol*. **37**(3):534-542. doi: 10.1161/ATVBAHA.116.308715.
21. Cao, Q. et al. (2016). Myeloid deletion of α1AMPK exacerbates atherosclerosis in LDL receptor knockout (LDLRKO) mice. *Diabetes.* **65**:1565-1576.
22. Huang, M. et al. (2015). Niclosamide inhibits the inflammatory and angiogenic activation of human umbilical vein endothelial cells. *Inflamm Res.* **64**:1023-1032.
23. Shah, D. et al. (2015). C1q deficiency promotes pulmonary vascular inflammation and enhances the susceptibility of the lung endothelium to injury. *J Biol Chem*. doi:10.1074/jbc.M115.690784.
24. Campos-Estrada, C. et al. (2015). Simvastatin and benznidazole-mediated prevention of Trypanosoma cruzi-induced endothelial activation: role of 15-epi-lipoxin A4 in the action of simvastatin. **9**:e0003770.
25. Ibrahim, A. S. et al. (2015). A lipidomic screen of hyperglycemia-treated HRECs links 12/15-Lipoxygenase to microvascular dysfunction during diabetic retinopathy via NADPH oxidase. *J Lipid Res*. **56**:599-611.
26. Cao, Q. et al. (2014). Inhibiting DNA Methylation by 5-Aza-2'-deoxycytidine ameliorates atherosclerosis through suppressing macrophage inflammation. *Endocrinology.* **155**:4925-4938.
27. Cary, L. H. et al. (2014). Interactions between endothelial cells and T cells modulate responses to mixed neutron/gamma radiation. *Radiat Res.* **181**:592-604.
28. Burrows, G. G. et al. (2014). Human multipotent adult progenitor cells transcriptionally regulate fucosyltransferase VII. *Cytotherapy.* **16**:566-575.
29. Kapitsinou, P. P. et al. (2014). Endothelial HIF-2 mediates protection and recovery from ischemic kidney injury. *J Clin Invest.* **124**:2396.
30. Wu, X. Y. et al. (2014). Regulation of microRNA-155 in endothelial inflammation by targeting nuclear factor (NF)-κB p65. *J Cell Biochem.* **115**:1928-1936.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com