
Product Manual

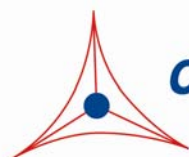
CytoSelect™ Leukocyte-Endothelium Adhesion Assay

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 $\beta 2$ 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.

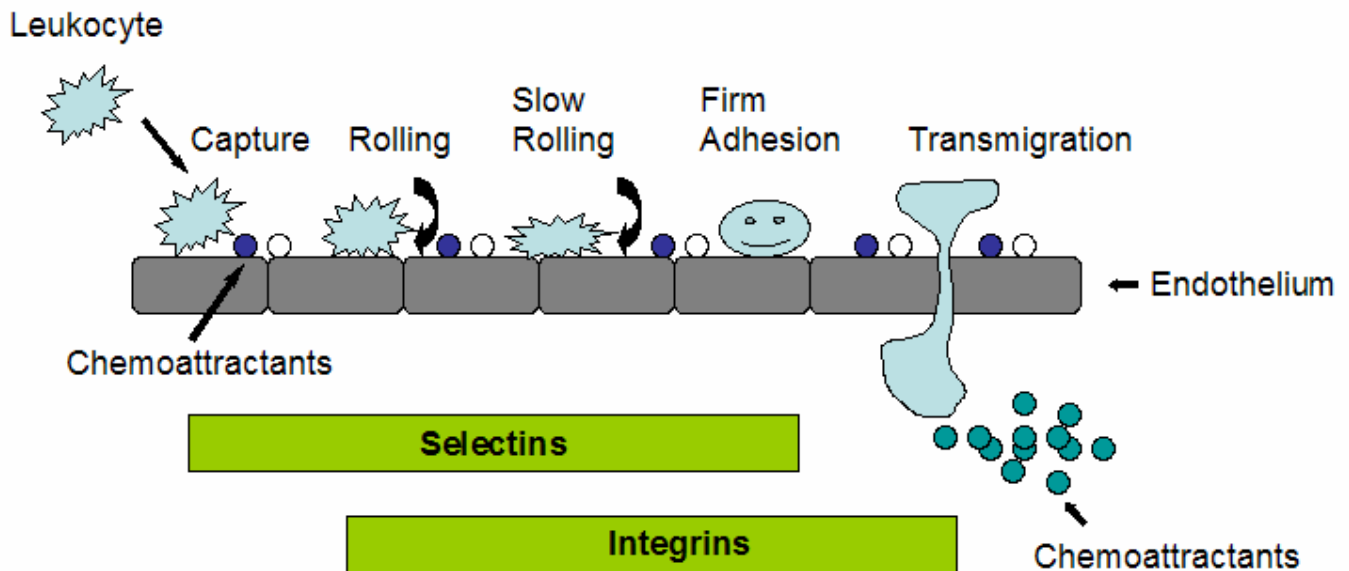
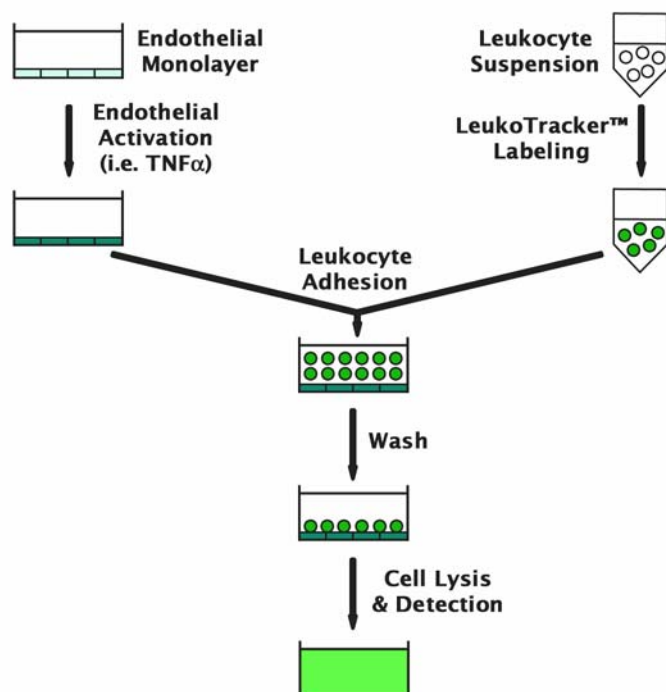


Figure 1. The Leukocyte-Endothelium Adhesion Cascade.

Assay Principle



Related Products

1. CBA-211: CytoSelect™ Leukocyte-Epithelium Adhesion Assay
2. CBA-215: CytoSelect™ Tumor-Endothelium Adhesion Assay
3. CBA-212: CytoSelect™ Leukocyte Transmigration Assay
4. CBA-216: CytoSelect™ Tumor Transendothelial Migration Assay

Kit Components

1. 500X LeukoTracker™ Solution (Part No. 12101): One Tube – 100 μL
2. Gelatin Solution (Part No. 12102): One Bottle – 12.0 mL of sterile 0.1 % Gelatin in 1X PBS
3. 4X Lysis Buffer (Part No. 10404): One Bottle – 10.0 mL
4. 10X Wash Buffer (Part No. 12104): One Bottle – 20.0 mL
5. $\text{TNF}\alpha$ (Part No. 12105): One Tube – 100 μL of 10 $\mu\text{g/mL}$ $\text{TNF}\alpha$ in sterile 1X PBS/0.1%BSA

Materials Not Supplied

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

Storage

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

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 48-well tissue culture treated plate, or 100 µL of the Gelatin Solution to each well of 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 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 1.0×10^6 cells/ml in serum free media. Add LeukoTracker to a final concentration of 1X (for example, add 2 μ L 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. Resuspend the cell pellet at $0.25 - 1.0 \times 10^6$ cells/ml in serum free media.
6. Aspirate endothelial culture media and wash once with serum free media. Add 200 μ L 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 μ L 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 μ L of 1X Lysis Buffer to each well containing cells. Incubate 5 minutes at room temperature with shaking.
11. Transfer 100 μ 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™ 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.

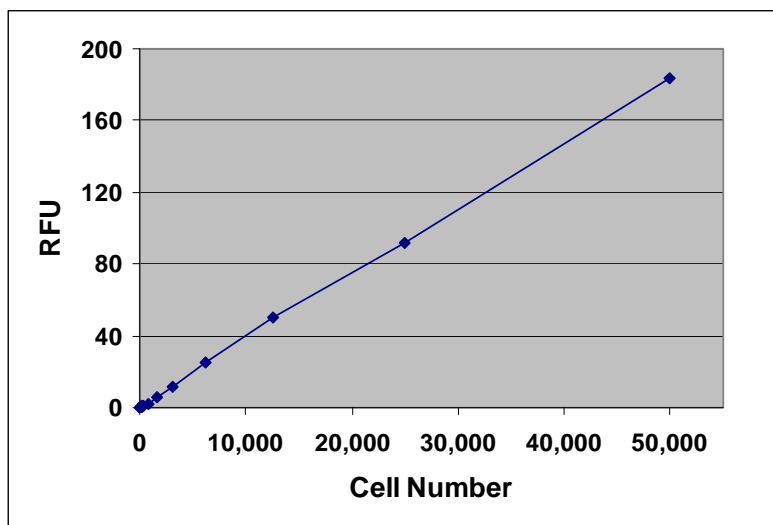


Figure 2. Quantitation of Human Monocytic THP-1. LeukoTracker™ labeled THP-1 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 μ L of cell suspension was mixed with 75 μ L of 2X Lysis Buffer). Fluorescence was quantified as described in Assay Protocol.

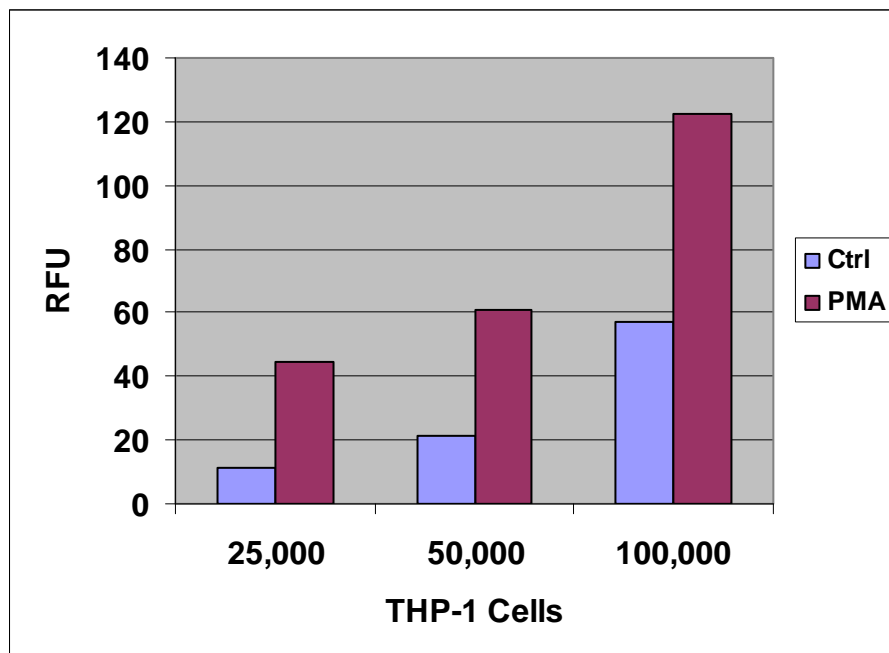


Figure 3. Human Monocytic THP-1 Adhesion to HUVEC Monolayer. HUVEC monolayer in 48-well plate was treated with 1 μ M PMA for 12 hrs. LeukoTracker™ labeled THP-1 cells were allowed to attach to HUVEC monolayer for 1 hr. Adherent cells were lysed and quantified by as described in the Assay Protocol.

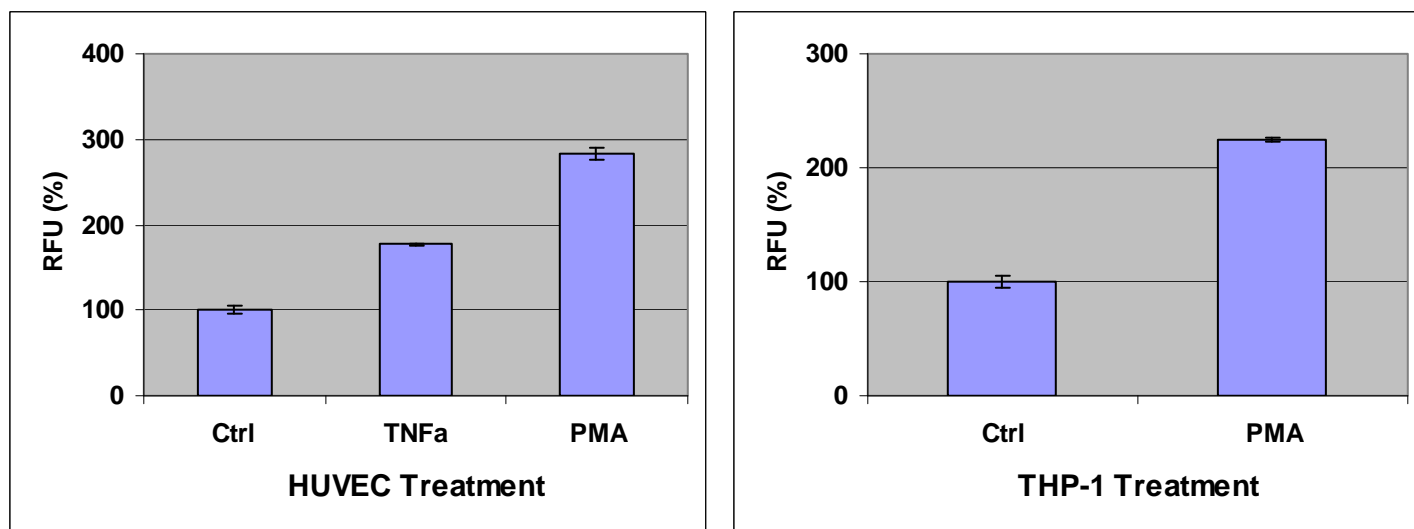


Figure 4. Cytokine Activation of Human Monocytic THP-1 Adhesion to HUVEC. HUVEC monolayer (left) or THP-1 cells (right) were treated with 50 ng/mL TNF α or 1 μ M PMA for 12 hrs. LeukoTracker™ labeled THP-1 cells (50,000 cells/well) were allowed to attach to HUVEC monolayer for 1 hr. Adherent cells were 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. Xue, J. et al. (2009). NFkB regulates thrombin-induced ICAM-1 gene expression in cooperation with NFAT by binding to the intronic NFkB site in the ICAM-1 gene. *Physiol. Genomics* 10.1152/physiolgenomics.00012.2009.

Warranty

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