

**NOTE: Revision to
Assay Protocol**

Product Manual

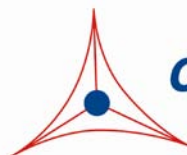
CytoSelect™ Leukocyte Transmigration Assay

Catalog Number

CBA-212

24 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 Transmigration Assay provides a robust system for the quantitative determination of leukocyte-endothelium interactions and transigrations. The kit contains sufficient reagents for the evaluation of 24 assays in a 24-well plate.

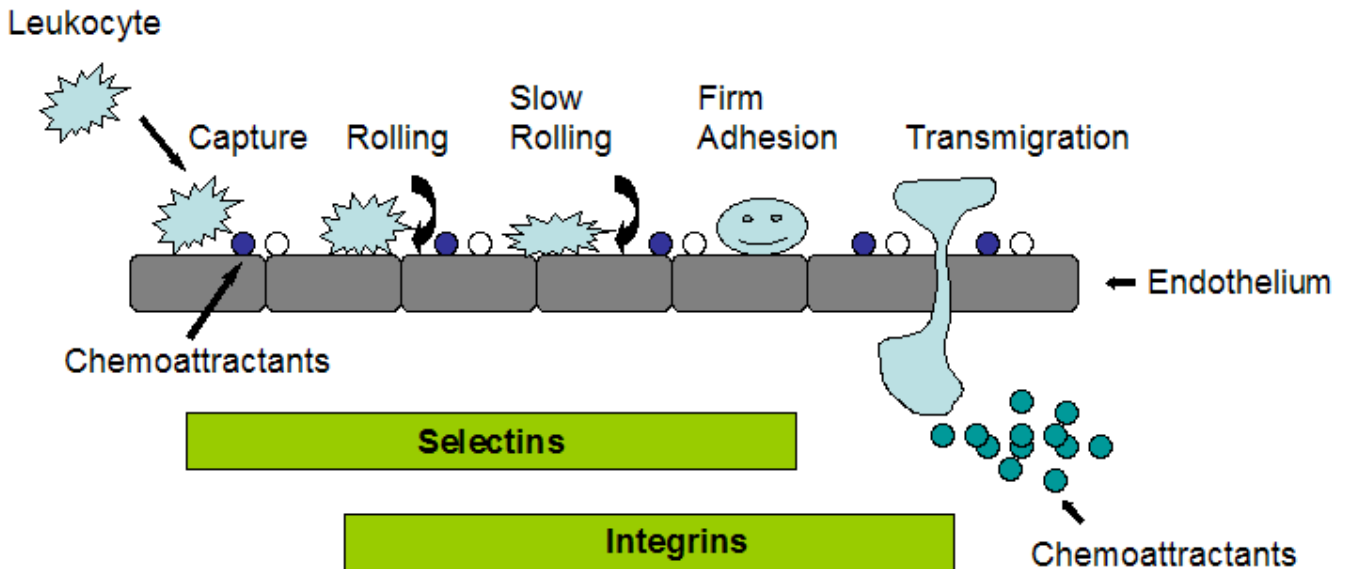
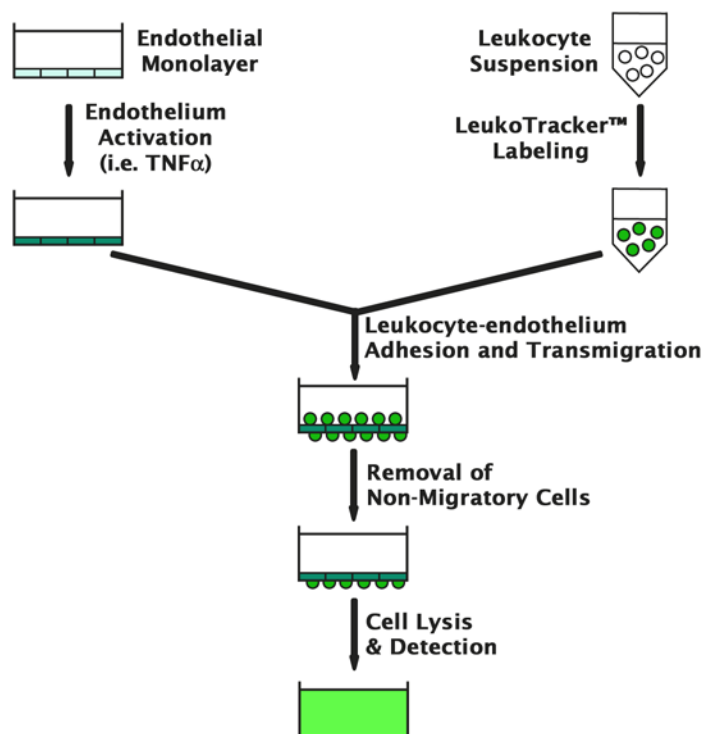


Figure 1. The Leukocyte Adhesion Cascade

Assay Principle



Related Products

1. CBA-070: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Colorimetric)
2. CBA-071: CytoSelect™ 48-Well Cell Adhesion Assay (ECM Array, Fluorometric)
3. CBA-100: CytoSelect™ 24-Well Cell Migration Assay (8 μ m, Colorimetric)
4. CBA-101: CytoSelect™ 24-Well Cell Migration Assay (8 μ m, 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-215: CytoSelect™ Tumor-Endothelium Adhesion Assay
11. CBA-216: CytoSelect™ Tumor Transendothelial Migration Assay

Kit Components

1. 24-well Migration Plate (Part No. 121201): One 24-well plate containing 24 cell culture inserts (3 μ m pore size)
2. 500X LeukoTracker™ Solution (Part No. 12101): One 100 μ L tube

3. 4X Lysis Buffer (Part No. 10404): One 10 mL bottle
4. TNF α (Part No. 12105): One 100 μ L tube of 10 μ g/mL TNF α 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 tissue culture plate
3. Serum free medium, such as RPMI 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
8. Fluorescence plate reader

Storage

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

Assay Protocol

1. Add 50,000-100,000 endothelial cells in 100 μ L medium to each insert in a 24-well plate containing 500 μ L 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 TNF α .
4. Harvest leukocytes and prepare a cell suspension at 0.5 - 1.0 x 10⁶ cells/ml in serum free media.
5. 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). 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 x 10⁶ 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 μ L of leukocyte culture media including desired chemoattractant(s).
7. Add 100 μ L 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.
Note: Retain the medium in the 24-well migration plate that contains chemoattractant(s) and cells that migrated through the membrane and into the medium.
10. Transfer 400 μ L of the 500 μ L bottom medium solution containing migratory cells (step 9) to a clean well that contains 150 μ L of 4X Lysis Buffer and place the swabbed insert into the same well. Incubate 5 minutes at room temperature with shaking.
Note: This step combines cells that migrated through the membrane and into the medium, and migratory cells still attached to the bottom side of the membrane.
11. Transfer 150 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 Transmigration 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.

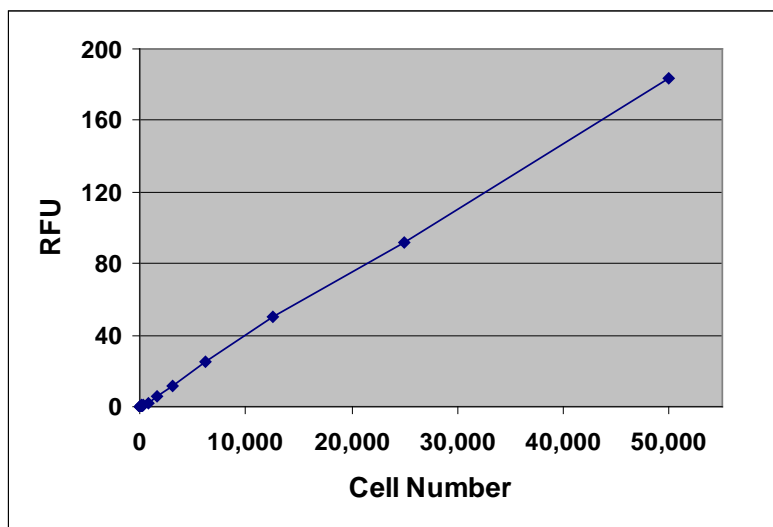


Figure 2. Quantitation of Human Monocytic THP-1. LeukoTracker™ labeled THP-1 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 μ L of cell suspension was mixed with 75 μ L of 2X Lysis Buffer). Fluorescence was quantified as described in Assay Protocol.

References

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Recent Product Citations

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5. Giunzioni, I. et al. (2014). Cigarette smoke condensate affects monocyte interaction with endothelium. *Atherosclerosis.* **234**:383-390.
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Warranty

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