Product Manual

CytoSelect™ Leukocyte-Epithelium Adhesion Assay

Catalog Number

CBA-211 **100 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

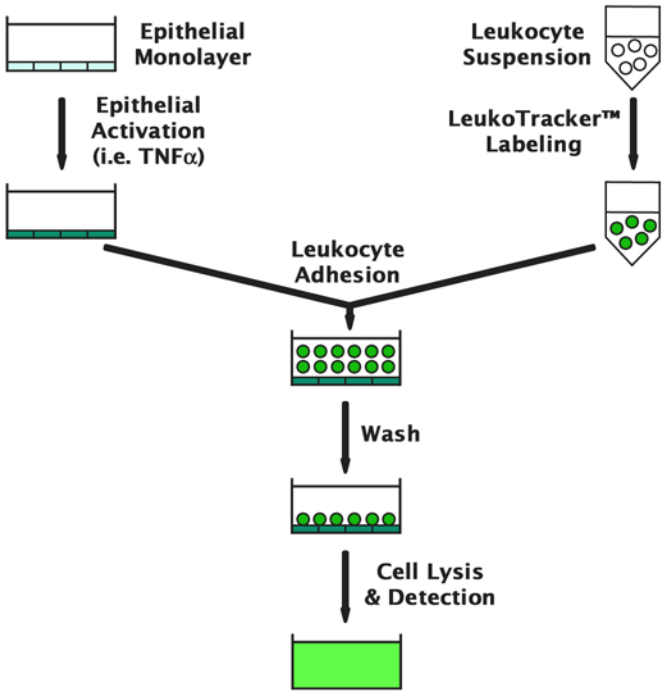
CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Airway inflammation is a hallmark of numerous lung diseases including chronic bronchitis, asthma, pulmonary fibrosis, and adult respiratory distress syndrome. The recruitment of circulating inflammatory cells into the airways involves both cell-cell adhesion molecules and soluble mediators. Accumulation of polymorphonuclear leukocytes (PMN) in the airway depends first on transmigration across the vascular endothelium. Once PMN is beyond the endothelial barrier, chemotactic signals frequently lead to the accumulation of these cells near mucosal epithelial cells as well as in the lumen of the airway. Airway epithelial cells have been shown to be an abundant source of chemokines for PMN under appropriate stimulatory conditions. These epithelial cells also express intercellular adhesion molecule (ICAM)-1, an important adhesive ligand for PMN. Airway epithelial cells may therefore be important not only for retention and activation of PMN, but also in the passage of these cells across epithelial cells and into the airway itself.

Cell Biolabs' CytoSelect™ Leukocyte-epithelium Adhesion Assay provides a robust system for the quantitative determination of leukocyte-epithelium interactions. The kit contains sufficient reagents for the evaluation of 100 assays in a 96-well plate.

Assay Principle



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-210: CytoSelect™ 96-Well Leukocyte-endothelium Adhesion Kit
7. CBA-215: CytoSelect™ Tumor-Endothelium Adhesion Assay
8. CBA-212: CytoSelect™ Leukocyte Transmigration Assay
9. CBA-216: CytoSelect™ Tumor Transendothelial Migration Assay
10. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

Kit Components

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

Materials Not Supplied

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

Assay Protocol

1. Add 50,000-100,000 epithelial cells/well to the 48-well or 96-well plate.
2. Culture cells for 48-72 until the epithelial cells form a monolayer.
3. Treat epithelial 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 epithelial culture media and wash once with serum free media. Add 200 µL of the leukocyte cell suspension to each well already containing the epithelial 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-epithelium 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.

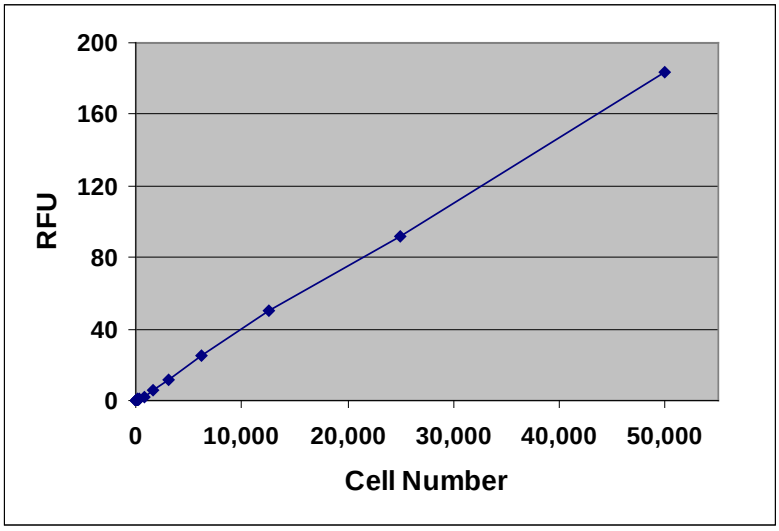


Figure 1. Quantitation of Human Monocytic THP-1. LeukoTracker™ labeled THP-1 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 µL of cell suspension was mixed with 75 µL of 2X Lysis Buffer). Fluorescence was quantified as described in Assay Protocol.

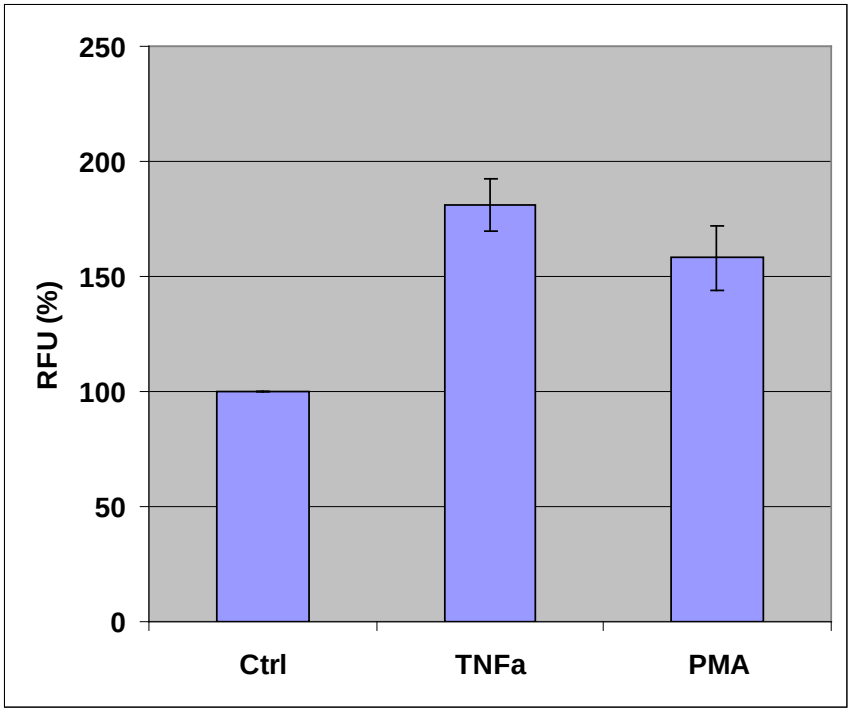


Figure 2. Human Monocytic THP-1 Adhesion to Cytokine-activated A549 Monolayer . A549 monolayer was treated with 50 ng/mL TNFα or 1 µM PMA for 12 hrs. LeukoTracker™ labeled THP-1 cells (50,000 cells/well) were allowed to attach to the A549 monolayer for 1 hr. Adherent cells were lysed and quantified by as described in the Assay Protocol.

References

1. Zen K. and Parkos C. A. (2003) *Curr Opin Cell Biol.* **15**, 557-64.

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.

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