Product Manual

# CytoSelect™ Tumor-Endothelium Adhesion Assay

Catalog Number

CBA-215 100 assays

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

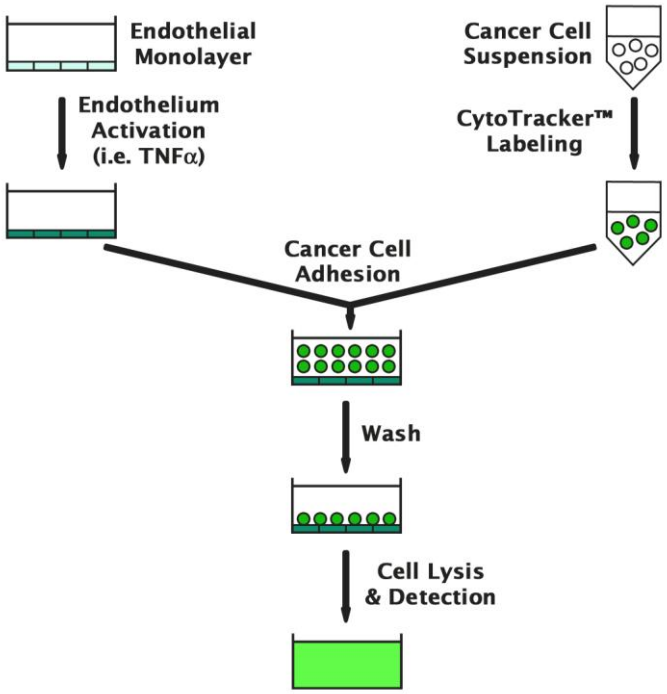
## Introduction

Cancer metastasis comprises several steps. First tumor cells are shed into the blood stream (intravasation), survive while traveling through the blood vessels, and finally migrate again out of the vessels (extravasation) into a new location in the body.

The initial arrest and attachment of tumor cells to vascular endothelium precedes their extravasation from the blood stream and is a crucial step in the tumor metastatic cascade. Tumor cell extravasation is equivalent, in many respects, to the entry of leukocytes into inflammatory tissue. Leukocyte extravasation consists of multiple, consecutive processes including the capture of circulating leukocytes, subsequent leukocyte rolling, arrest, firm adhesion and transmigration. Increasing evidence suggests that tumor cell adhesion to the endothelial lining is influenced by endothelial activation or tissue-specific differences in endothelium and depends on the expression of specific cell surface molecules. E-Selectin and Vascular Cell Adhesion Molecule-1 (VCAM-1) appear to play a pivotal role in the tumor-EC interaction.

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

## 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-150: CytoSelect™ 96-Well In Vitro Tumor Sensitivity Assay
4. CBA-155: CytoSelect™ Clonogenic Tumor Cell Isolation Kit
5. CBA-210: CytoSelect™ Leukocyte-Endothelium Adhesion Assay
6. CBA-211: CytoSelect™ Leukocyte-Epithelium Adhesion Assay
7. CBA-212: CytoSelect™ Leukocyte Transmigration Assay
8. CBA-216: CytoSelect™ Tumor Transendothelial Migration Assay
9. CBA-320: CytoSelect™ 96-Well Hematopoietic Colony Forming Cell Assay

## Kit Components

1. 500X CytoTracker™ Solution (Part No. 12151): One 100 µL tube
2. Gelatin Solution (Part No. 12102): One 10 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. 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

CytoTracker™ 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 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 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 cancer cells with desired activator or inhibitor for 6-12 hrs.
4. Harvest cancer cells and prepare a cell suspension at 1.0 x $10^6$ cells/ml in serum free media. Add CytoTracker™ to a final concentration of 1X (for example, add 2 µL of 500X CytoTracker™ solution to 1.0 mL of cancer 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 cancer 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 cancer cells 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™ Tumor-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.

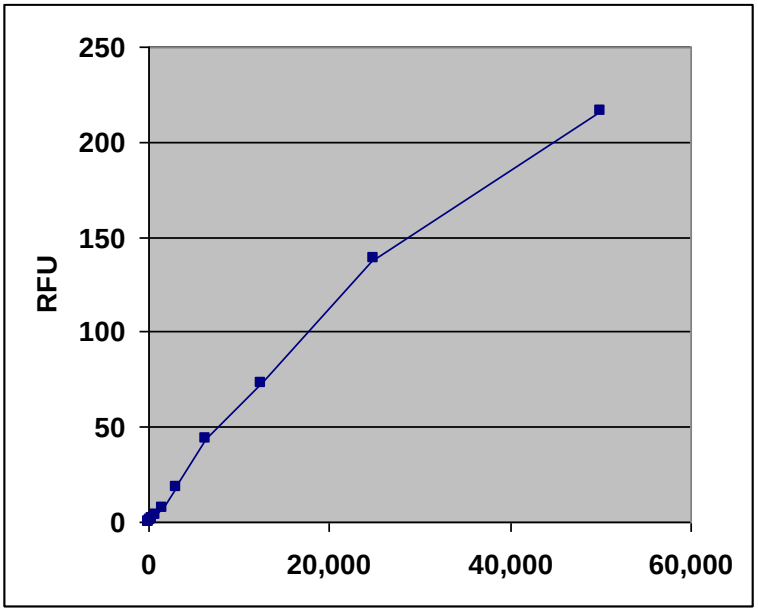
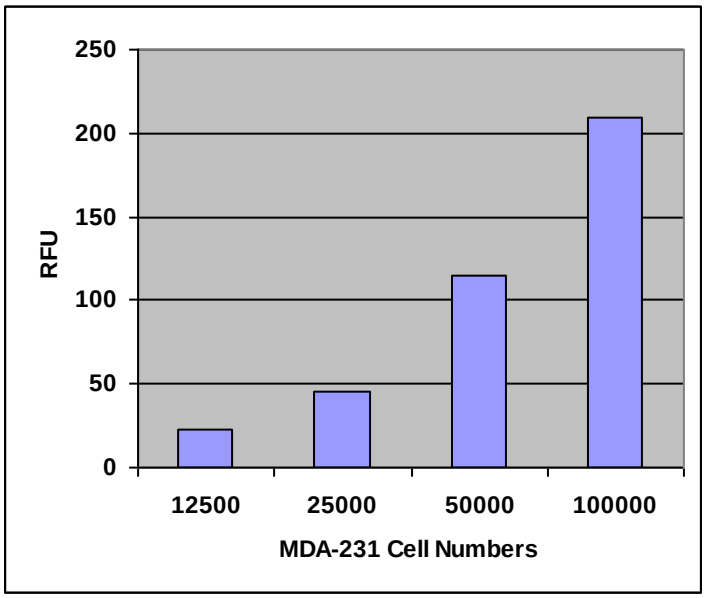



**Figure 1. Quantitation of Human Breast Cancer MDA-231 Cells**. CytoTracker™ labeled MDA-231 cells were titrated in 1X PBS, then subsequently lysed with 2X Lysis Buffer (75 µL of cell suspension was mixed with 75 µL of 2X Lysis Buffer). Fluorescence was quantified as described in the Assay Protocol.



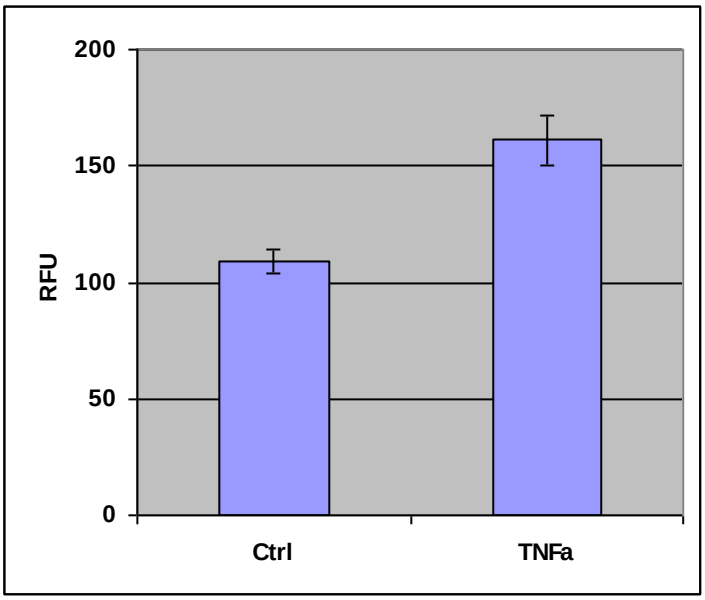




**Figure 2. Human Breast Cancer MDA-231 Adhesion to HUVEC Monolayer. Left:** CytoTracker™ labeled MDA-231 cells were allowed to attach to HUVEC monolayer in 48-well plate for 1 hr. Adherent cells were lysed and quantified by as described in Assay Protocol. **Right:** HUVEC monolayer in 48-well plate was treated with 50 ng/mL TNFα for 12 hrs. CytoTracker™ labeled MDA-231 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 Assay Protocol.

## References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## Recent Product Citations


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