ab325586 – Leukocyte-endothelium Adhesion Assay

A robust system for the quantitative determination of leukocyte-endothelium interactions.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325586

Storage and Stability: 500X Tracker Solution and TNF-alpha should be removed from the kit and stored at -20°C immediately. Store all other components at 4°C. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

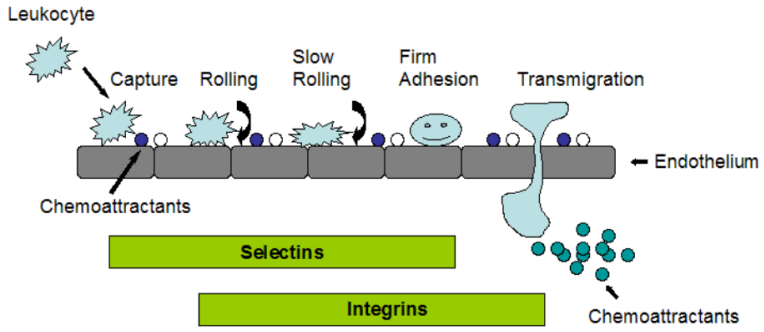
Item	Quantity 100 Tests	Storage Condition
4X Lysis Solution	10 mL	+4°C
500X Tracker Solution	100 µL	-20°C
Gelatin Solution	12 mL	+4°C
10X Wash Buffer	20 mL	+4°C
TNF-alpha	100 µL	-20°C

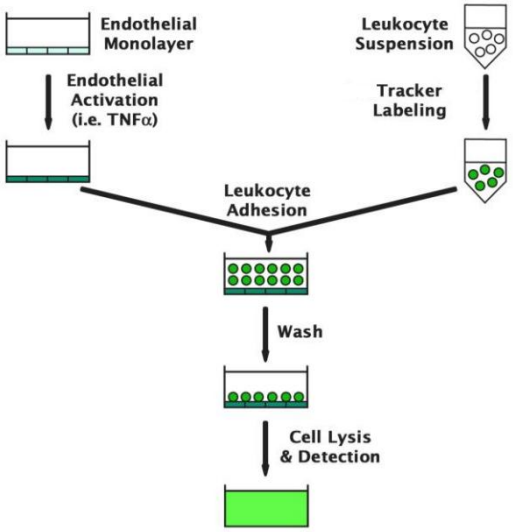
Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

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

The Leukocyte-Endothelium Adhesion Cascade





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 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 hrs 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 Tracker to a final concentration of 1X (for example, add 2 µL of 500X Tracker 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 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.

Technical Support

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For all technical or commercial enquiries please go to:
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https://www.abcam.cn/contact-us (China)
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