

ab325587 – Leukocyte Transmigration Assay

A robust system for the quantitative determination of leukocyte-endothelium interactions and transigrations.

For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325587

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

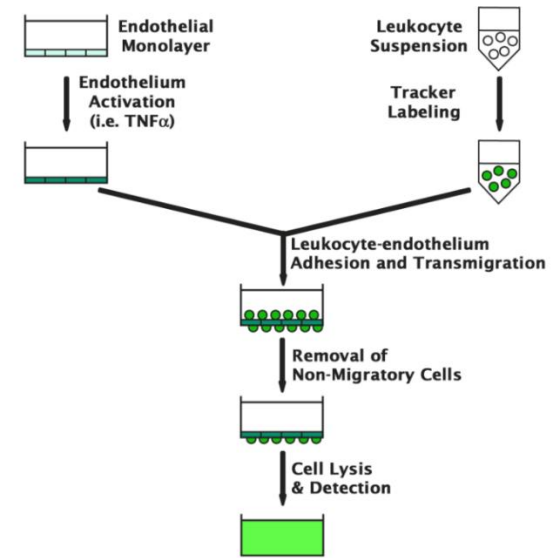
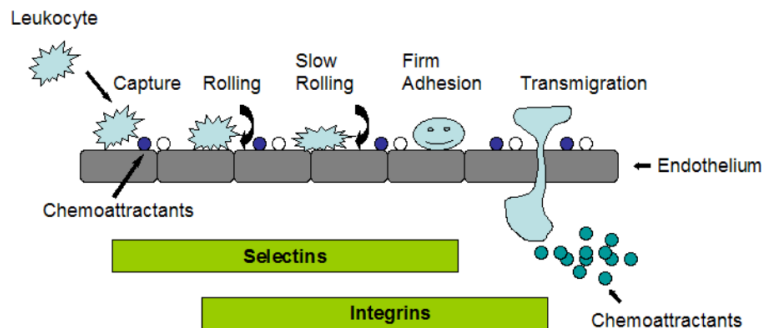
Item	Quantity 24 Tests	Storage Condition
4X Lysis Solution	10 mL	+4°C
Cotton Swabs	1 pack	+4°C
Forceps	1 unit	+4°C
500X Tracker Solution	100 µL	-20°C
TNF-alpha	100 µL	-20°C
24-Well Migration Plate	2 x 1 unit	+4°C

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
- 24-well tissue culture plate
- Serum free medium, such as RPMI containing 0.5% BSA, 2 mM CaCl₂ and 2 mM MgCl₂
- Cell culture incubator (37°C, 5% CO₂ atmosphere)
- 1X PBS containing 2 mM CaCl₂ and 2 mM MgCl₂
- Light microscope
- 96-well plate suitable for a fluorescence plate reader
- Fluorescence plate reader

The Leukocyte-Endothelium Adhesion Cascade



Assay Protocol

1. Add 50,000-100,000 endothelial cells in 100 µL medium to each insert in a 24-well plate containing 500 µL of culture medium.
2. Culture cells for 48-72 hrs until the endothelial cells form a monolayer.
3. Treat endothelial cell monolayer with desired activator or inhibitor, such as TNF-alpha.
4. Harvest leukocytes and prepare a cell suspension at 0.5 - 1.0 x 10⁶ cells/mL in serum free media.
5. Add Tracker solution to a final concentration of 1X (for example, add 2 µL of 500X Tracker 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 µL of leukocyte culture media including desired chemoattractant(s).
7. Add 100 µL 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.

ΔNote: 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.
 Δ 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.

Technical Support

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