ab325588 – Tumor Transendothelial Migration Assay

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

For overview, typical data and additional information please visit: www.abcam.com/ab325588

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
TNF-alpha	100 µL	-20°C
24-Well Migration Plate	1 unit	+4°C
500X Tracker Solution	100 µL	-20°C

Materials Required, Not Supplied

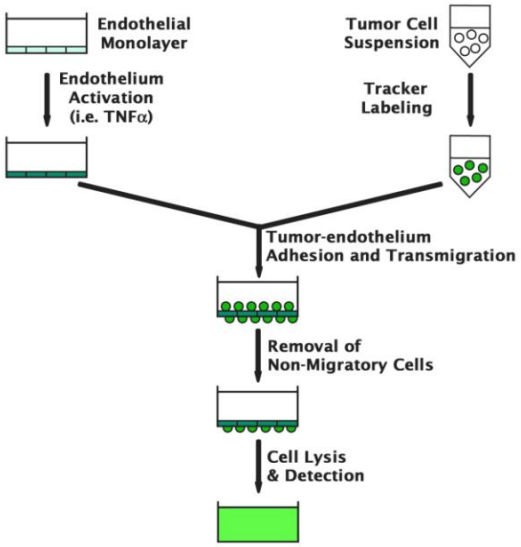
These materials are not included in the kit, but will be required to successfully utilize this assay:

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

Preparation of Reagents

- **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 Principal



Assay Protocol

1. Add 50,000-100,000 endothelial cells in 300 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 cancer cells and prepare a cell suspension at 0.5 - 1.0 x 10^6 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 cancer 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 10^6 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 tumor cell culture media including 10% fetal bovine serum or desired chemoattractant(s).
7. Add 300 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.

10. Transfer the insert to a clean well containing 200 µL of 1X Lysis Buffer. 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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