

ab325584 – Endothelial Tube Formation Assay (In Vitro Angiogenesis Assay)

A robust system to assess angiogenesis in vitro.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit: www.abcam.com/ab325584

Storage and Stability: Store kit at -20°C immediately upon receipt. Refer to list of materials supplied for storage conditions of individual components.

Materials Supplied

Item	Quantity 50 Tests	Storage Condition
ECM Gel Solution	0.5 mL	-20°C
10X Staining Buffer	1 mL	-20°C
Staining Dye	50 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 such as HUVEC, HMEC
- Endothelial cell culture medium
- 1XPBS
- 37°C Incubator, 5% CO₂ atmosphere
- Light microscope
- Fluorescence microscope
- (optional) Tube quantification software

Preparation of Reagents

- **ECM gel:** Thaw overnight on ice or in a frost-free 4°C refrigerator, store the thawed ECM gel solution up to 10 days, do not refreeze. The ECM gel solution will gel rapidly at room temperature. The tube MUST be kept on ice all the time.
- **1X Staining Buffer:** Prepare a 1X Staining Buffer by diluting the provided 10X stock 1:10 in dH₂O. Store the diluted solution at room temperature.
- **1X Staining Solution:** FRESHLY prepare 1X Staining Buffer by diluting the provided Staining Dye to 1 µM with 1X Staining Buffer. Store 1X Staining Solution at room temperature.

Assay Protocol

Tube Formation

1. Thaw ECM gel and prepare 1X Staining Buffer as described above.
2. Add 50 µL of thawed ECM gel solution to each well of a pre-chilled 96-well sterile plate.
ΔNote: Undiluted ECM gel solution is very viscous and quickly gels at room temperature, so it MUST be kept on ice all the time. When pipetting, use a pre-chilled pipette tip to prevent any ECM gel formation during transfer.
3. Incubate 30 minutes to 1 hr at 37°C to allow the ECM solution to form a gel.
4. Harvest endothelial cells and resuspend in desired culture medium containing 0.5-10% serum and your desired angiogenesis mediators at 1-2 x 10⁵ cells/mL.
5. Add 150 µL of cell suspension (1.5-3 x 10⁴ cells) per well onto the solidified ECM gel. Incubate the assay plate at 37°C for 4 to 18 hrs.
6. Examine the endothelial tubes using light microscope in high magnification field. It is possible to visually estimate the extent of tube formation by inspecting the overall tube length and branch points.

Fluorescence Labeling

1. Carefully remove medium by gently blotting on paper towels. Be carefully not to disturb endothelial tubes.
2. Gently wash with 100 µL of 1X Staining Buffer and remove the wash as described in step 1.
3. Add 50 µL of 1X Staining Solution per well and incubate 30 min at 37°C.
4. Gently wash with 100 µL of 1X PBS and remove the wash as described in step 1. Repeat this step twice.
5. Examine endothelial cells and tubes using a fluorescence microscope. Acquire several images per well and process them using imaging analysis software such as NIH Image or Image Pro Plus.
ΔNote: Calcein AM-labeled tubular structure is stable for only 1-2 hrs in 1X PBS at 4°C.

Technical Support

Copyright © 2026 Abcam, All Rights Reserved. The Abcam logo is a registered trademark. All information / detail is correct at time of going to print.

Version 1 | 2026-03-11

For all technical or commercial enquiries please go to:

<https://www.abcam.com/en-us/contact-us>

<https://www.abcam.cn/contact-us> (China)

<https://www.abcam.co.jp/contact-us> (Japan)