Product Manual

Endothelial Tube Formation Assay (In Vitro Angiogenesis)

Catalog Number

CBA-200 **50 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Angiogenesis, or neovascularization, is the process of generating new blood vessels derived as extensions from the existing vasculature. The principal cells involved are endothelial cells, which line all blood vessels and constitute virtually the entirety of capillaries. Angiogenesis involves multiple steps; to achieve new blood vessel formation, endothelial cells must first escape from their stable location by breaking through the basement membrane. Once this is achieved, endothelial cells migrate toward an angiogenic stimulus such as might be released from tumor cells, or wound-associated macrophages. In addition, endothelial cells proliferate to provide the necessary number of cells for making a new vessel. Subsequent to this proliferation, the new outgrowth of endothelial cells needs to reorganize into a three-dimensionally tubular structure. Each of these elements, basement membrane disruption, cell migration, cell proliferation, and tube formation, can be a target for intervention, and each can be tested *in vitro* and in *vivo*.

Several *in vivo* assay systems including the chick chorioallantoic membrane (CAM) assay, an in vivo Matrigel plug assay, the corneal angiogenesis assay have been developed that permit a more realistic appraisal of the angiogenic response. However, all of them are time consuming, tedious and require technical specialties.

One quick assessment of angiogenesis is the measurement of the ability of endothelial cells to form three-dimensional structures (tube formation). Endothelial cells form tubes on collagen or fibrin clot coated dishes after several days. Cell Biolabs Endothelial Tube Formation Assay Kit utilizes ECM gel prepared from Engelbreth–Holm–Swarm (EHS) tumor cells and tube formation can be achieved within 18 hr. Endothelial tube formation on ECM gal is reasonably faithful to the *in vivo* situation. It can be used to test angiogenesis inhibitor before *in vivo* testing.

Cell Biolabs Endothelial Tube Formation Assay Kit provides a robust system to assess angiogenesis *in vitro*. Following staining the tube with the provided fluorescence dye, the extent of tube formation, such as average tube length and branch point, can be quantified through imaging software. Each kit provides sufficient quantities to perform up to 50 assays in 96-well plates.

Kit Components

1. ECM Gel Solution (Part No. 10010): Five tubes – 0.5 mL each, Ready-To-Use
2. 10X Staining Buffer (Part No. 10011): One tube – 1.0 mL
3. Staining Dye (Part No. 10012): One tube – 50 µL of 1 mM Calcein AM in DMSO

Materials Not Supplied

1. Endothelial cells such as HUVEC, HMEC
2. Endothelial cell culture medium
3. 1XPBS
4. 37ºC Incubator, 5% CO_2 atmosphere

5. Light microscope
6. Fluorescence microscope
7. (optional) Tube quantification software

Storage

Store all components at -20ºC until their expiration dates.

Preparation of Reagents

- ECM gel: Thaw overnight on ice or in a frost-free 4ºC 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 dH_2O. 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

I. 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.

   Note: 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 37^0C 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 10^5 cells/mL.
5. Add 150 uL of cell suspension (1.5-3 x 10^4 cells) per well onto the solidified ECM gel. Incubate the assay plate at 37^0C 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.

II. 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 37ºC.
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.

Note: Calcein AM-labeled tubular structure is stable for only 1-2 hrs in 1X PBS at 4ºC

Example of Results

The following figures demonstrate tube forming results with HUVEC cells using the Endothelial Tuber Formation Assay. One should use the data below for reference only. This data should not be used to interpret actual results.

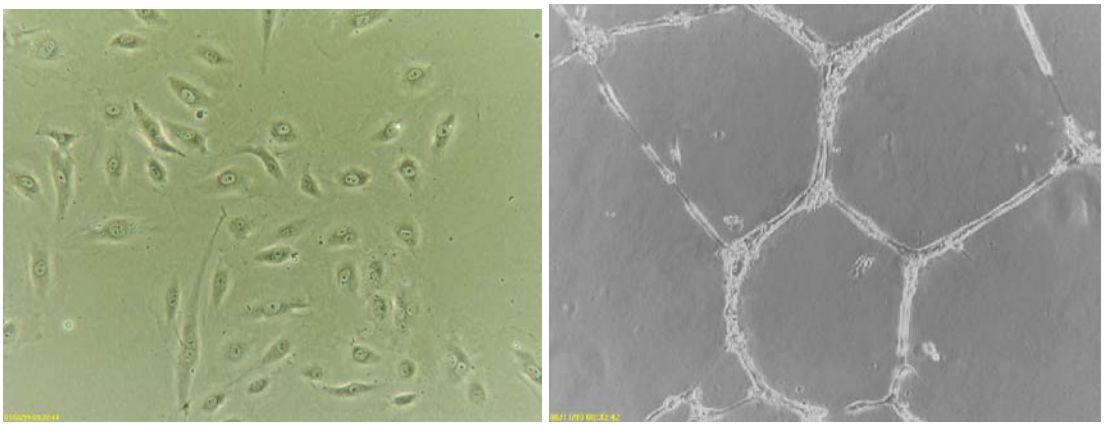

Figure 1. HUVEC Tube Formation on ECM Gel. Left: HUVEC cells on tissue culture plate. Right: Tube formation on ECM Gel.

References

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Recent Product Citations

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