ScienCell™
Research Laboratories

***In Vitro* Tube Formation Assay**
Cat. No. 8158, 50 tests

Introduction

Angiogenesis, or the formation of new blood vessels, plays an important role in many physiological (e.g. wound healing) and pathological (e.g. tumor growth and inflammation disease) processes. As a complex multi-step process, angiogenesis involves degradation of basement membrane, proliferation and migration of endothelial cells into extracellular matrix (ECM), followed by alignment and reorganization of endothelial cells into three-dimensional tubular structure and formation of new basement membrane. Specifically, the critical step of the formation of three-dimensional tubular structure by endothelial cells is the objective of many *in vivo* and *in vitro* angiogenesis assays to screen efficacy of both pro- and antiangiogenic agents.

The ScienCell™ In Vitro Tube Formation Assay provides a convenient system to measure the formation of three-dimensional tubular structure by enodothelial cells when cultured on an ECM gel of basement membrane extract. The ECM gel, which is prepared from Engelbreth Holm-Swarm (EHS) sarcoma produced in mice, consists of laminin, collagen IV, heparan sulfate proteoglycan, entactin and other minor components optimized for tube formation. With this system, tube formation can be achieved within 6-18 hours, with the earliest signs of tubular structure shown up after 3-4 hours. The Assay also provides a colorimetric cell staining reagent for better examination.

Kit Components

Cat. No.	# of vials	Name	Quantity	Storage
8158a	1	ECM Gel Solution	2.5 ml	-20˚C
8158b	1	Colorimetric Staining Reagent	2.5 ml	2-8˚C

Quality Control

ScienCell™ Human umbilical vein endothelial cells (HUVECs) are cultured on ECM gel from EHS tumor cells for 2-18 hours in ScienCell™ Dulbecco's Modified Eagle Medium (DMEM) with 10% Fetal Bovine Serum (FBS). Angiogenic tube formation is characterized by staining colorimetrically (Figure 1).

Procedures

A. Preparation of ECM gel

1. Thaw appropriate volume of ECM Gel Solution overnight in a “frost-free” 4˚C refrigerator.
2. Add 50 µl of thawed ECM Gel Solution to each well of 96-well plate. Since the gel solution solidifies quickly at room temperature, we suggest use pre-cooled pipettes and plates. Incubate for at least one hour at 37˚C to allow the solution to solidify into a homogenous gel.

B. Culture of Cells

1. Harvest endothelial cells and resuspend in culture medium with or without target angiogenic mediators.
2. Seed 100 µl of endothelial cell suspension at 0.5-2×10^5 cells/ml into each well of the 96-well

plate with ECM gel. Culture the cells in a tissue-culture incubator for the desired period of time (6-18 hrs).

C. **Staining and examination of cells**

1. The development of tubular structure can be examined in-situ with a phase-contrast microscope.
2. For better contrast, cells can be properly fixed and stained colorimetrically as follows: Carefully remove culture medium from cells, fix the cells with 0.1% glutaraldehyde for 5 minutes and wash once with PBS*. Then stain cells with the Colorimetric Staining Reagent (50 µl per well) for 15 minutes at room temperature. Gently remove the staining solution and wash the cells with PBS. Examine the cells using a bright-field objective.

*Since the tubular structures formed on ECM gel are relatively fragile, it is suggested to remove any medium or buffer from the cells during the staining procedure by gently blotting the 96-well plate on paper towels to avoid damaging the tubular structures.

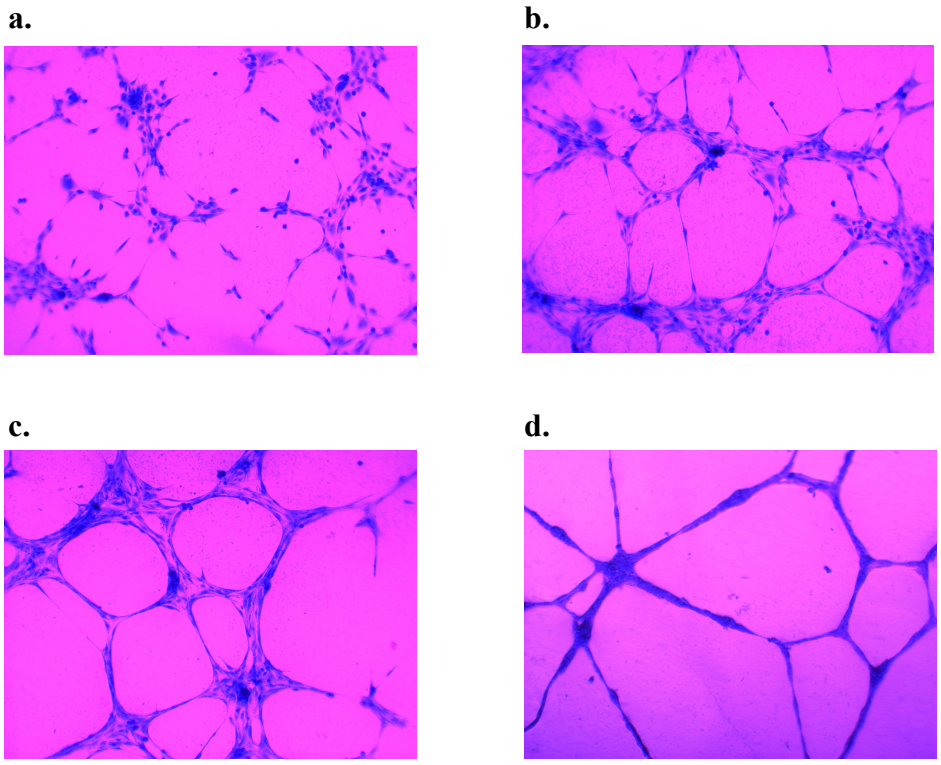

Figure 1. Development of tube-like structures by HUVECs cultured on ECM gel from EHS tumor cells after 2 hrs' (a), 4 hrs' (b), 8 hrs' (c) and 24 hrs' (d) of incubation.