THT Biomaterials GmbH
extracellular platform technology
The Human Touch

Assay Methods

Protocol: Endothelial Cell Tube Formation Assay

THT Biomaterials GmbH - extracellular platform technology

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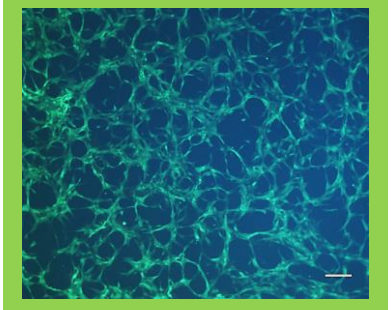

HUVEC on hpS after 2 days, scale bar=200 µm.

Introduction

Vasculogenesis is an essential event during development and characterized by a variety of cellular responses. In this manner, *in vitro* vasculogenesis assays are often used as a model for studying endothelial cell differentiation and tube formation.

For microscopic image analysis and quantification of the tubes, we recommend manual counting techniques, as false positive/negative search results in automated Software solutions are frequently observed.

Materials

- Human placenta Substrate; hpS®, order nr. THT0301
- 96 well flat-bottom standard tissue culture-treated plate (e.g. Greiner).
- Endothelial cell culture medium (e.g. EGM-2).
- Humidified tissue culture incubator, 37°C, 5% atmosphere.
- Endothelial cells (e.g. HUVEC).
- Laminar flow tissue culture hood.
- (Fluorescence) microscope.

Procedure

1. Reconstitution of hpS

1.1. Thaw hpS on ice.
1.2. Spray flask/vial with 70% ethanol and air dry.
1.3. Keep hpS on ice and handle using sterile techniques.
1.4. Resuspend hpS to evenly disperse the substrate.
1.5. Dispense hpS into appropriate working aliquots using pre-cooled pipet tips, vials or tubes, and refreeze hpS immediately.
1.6. Avoid multiple freeze thaws.

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2. Coating procedure

Note: once thawed, hpS should be used immediately. The protein concentration of hpS is batch-specific and can be found on the Certificate of Analysis.

2.1. Thaw hpS and resuspend it to homogeneity.
2.2. Add 50 µL hpS per plate (96 well). Avoid air bubbles.
2.3. Incubate the plates at 37°C for 1-3 hours.
2.4. The plates are now ready to use.

3. Endothelial Cell Tube Formation Assay

3.1. Prepare the well plate as described in section 2.
3.2. Culture endothelial cells (e.g. HUVEC) with desired endothelial cell medium to a confluence of around 80% (primary cells should have a passage number below 10).
3.3. Prepare endothelial cell suspension by trypsinizing the cell monolayer, centrifugation and resuspension in fresh culture medium including FCS, vasculogenic promoters or inhibitory agents (depending on the experimental setup).
3.4. Add 100 µL of cell suspension (+ e.g. 20.000 HUVEC cells) to each well.
3.5. Incubate the vasculogenesis assay plate at 37°C, 5% CO_2 atmosphere.
3.6. Replace culture medium every second day (the cell tubes should be stable around 5-7 days), Take microscopical images and analyze.

Note: Various researchers use an automated Software system to analyze different aspects of vasculogenesis assays (tube lengths, junctions, branch points or area). Automation may be timesaving, but significant differences in the results may occur. We recommend a blinded analysis by at least 2-3 persons using graphpad software and analyze the copy-pasted layer by an automated analysis software.