NEUROMICS

3D Human HUVECs Angiogenesis Model

Catalog #: 3D45001

GENERAL INFORMATION

Our 3D Human HUVECs Angiogenesis model is constructed using GFP-Tagged human umbilical vein endothelial cells (HUVECs) are co-cultured with RFP-Tagged supporting cells. GFP positive capillary like tubule formation can be monitored in real time under fluorescence microscope throughout the whole process of the experiment.

The 3D Human HUVECs Angiogenesis contains the materials necessary to preform multiple angiogenesis assays in 6, 12, or 24 well formats. The 3D model is designed that the testing materials, i.e. compounds, conditioned media, or tissue explants, can be added into the system at any time, ranging from the onset of vasculogenesis to advanced angiogenesis. The resulting effect on tubule formation (tubular length, number of branches, etc.) can be monitored throughout the whole process under inverted fluorescence microscope.

REAGENTS AND MATERIAL PROVIDED:

- 1 x 24, 12 or 6 well plate of seeded tissue in the insert
- 1 x 500ml of Endo-Growth Medium (4°C).

The 3D Human HUVECs Angiogenesis Model is a proprietary system in which GFP-tagged human endothelial cells from variable vascular beds are co-cultured with RFP-tagged human supporting cells in a specially designed medium. The endothelial cells initially form small islands within the culture matrix. They subsequently begin to proliferate and then enter a migratory phase during which they move through the matrix to form threadlike tubule structures with lumens. They gradually join up (by 1 - 2 weeks) to form a network of anastomosing tubules, which closely resembles the capillary bed found in vivo.

PROTOCOL:

Upon receiving the plate containing your cell:

1. Thaw your media. In a 37°C dry incubator. **Please avoid using a water bath to warm up Neutralization media.
2. Once the media reaches 37°C
3. Clean the bottle with 70%Ethanol
4. Place the media in a clean biosafety cabinet

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Thawing your plate:

1. Remove the plate from the box containing Dry Ice
2. Spray the plate with 70% Ethanol
3. Wipe the plate down and dry
4. Place in the same clean biosafety cabinet that contains your Neutralization Media

Adding Media To your Frozen plate:

1. Gently remove the cover from the frozen plate
2. **Be very careful not to touch the insert membrane**
3. Add 3ml of media into the top (insert) and bottom (wells) of frozen plate
4. Put the cover back on the plate
5. Transfer the plate to a 37°C containing 5% CO2 for two hours

After Two hours of incubation:

1. Open your incubator
2. Spray it down plate with 70% Ethanol, and wipe down
3. Place the plate into a clean biosafety cabinet
4. **Be very careful not to touch the insert membrane**
5. Pipet all the old media out from the plate
6. Add about 3ml of growth media to the top (insert) and bottom (wells) of the plate.
6. Transfer the plate to a 37°C containing 5% CO2
7. Change media every 48 hours until the plate becomes confluent

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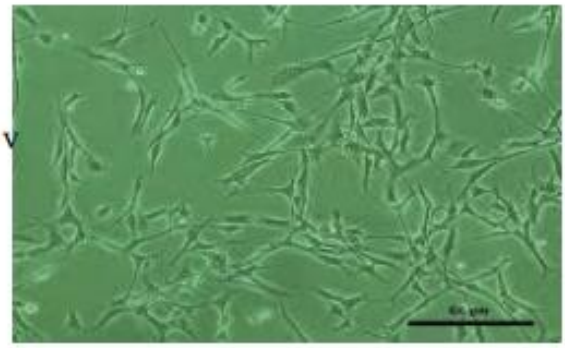
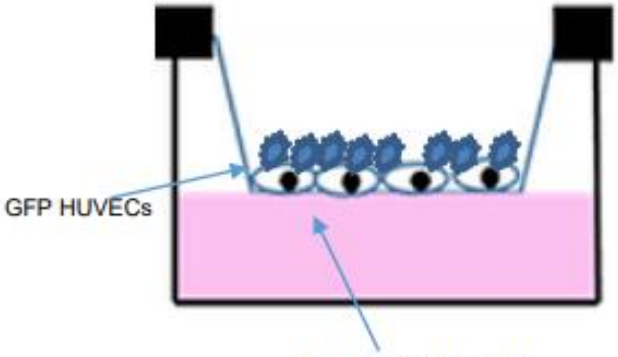
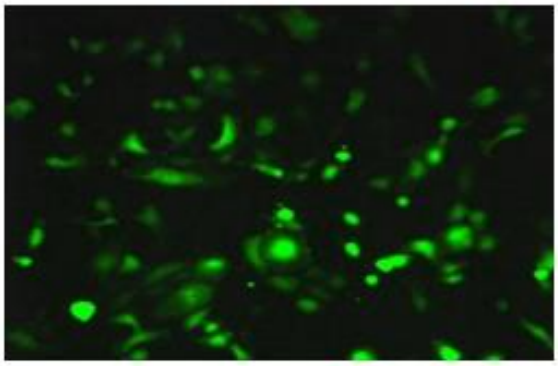
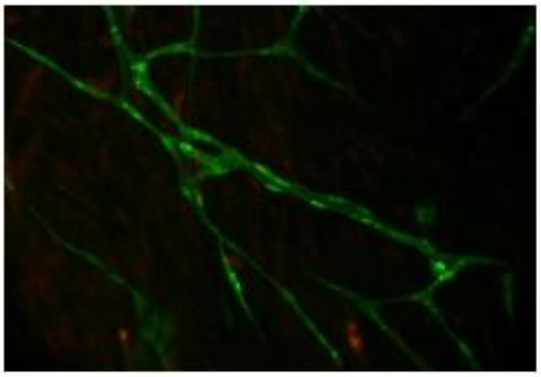


HUVECs growing in inserts

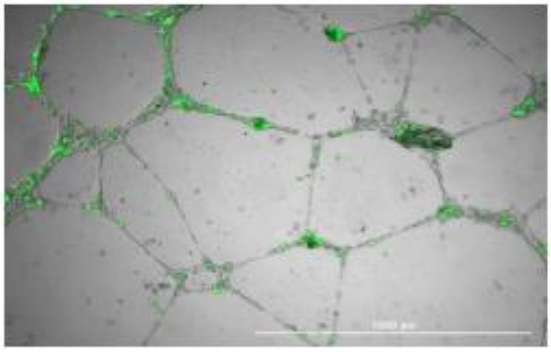




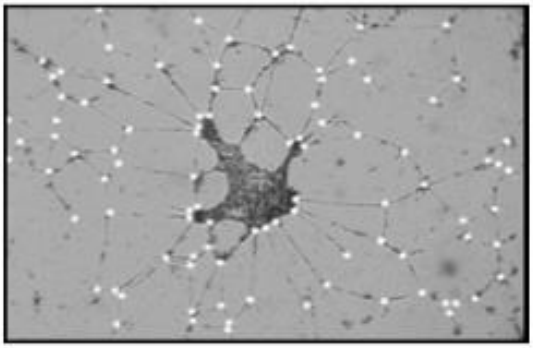
HUVECs before tube formation



HUVECs after tube formation



4x overlay image of HUVECs used in an endothelial cell capillary tube formation assay. The cells were stained with calcein. Imaging was performed in the brightfield and GFP channels



Co-culture system

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