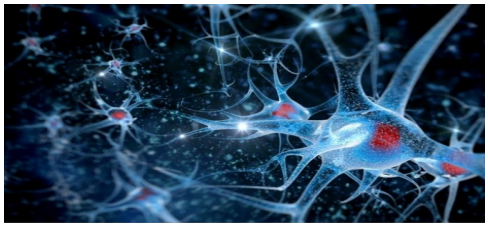
3D Human Retinal Microvascular Angiogenesis

Product Name	3D Human Retinal Microvascular Angiogenesis
Catalog Number	EP008
Product Format	6, 12, and 24 well
Storage	37°C



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GENERAL INFORMATION

Retinal microvascular morphogenesis is a complex and highly coordinated process, which occurs during embryonic development, post-natally and in association with several visually-impairing diseases, including retinopathy of prematurity (ROP), age-related macular degeneration and diabetic retinopathy.

Retinal vascular formation is a complex process that requires a precise temporospatial regulation of various elements, including the growth factors. Insulin-like growth factor-1 (IGF-1) and vascular endothelial growth factor (VEGF) are ligands of specific receptor tyrosine kinases (RTKs), which, if activated, will initiate downstream pathways that ultimately promote cell survival, cell proliferation, vascular permeability and cell migration; all of which permit blood vessel development. Formation of blood vessels from pre-existing vessels may be normal (angiogenesis) or pathological (neovascularization) processes.

Our 3D Human Retinal Microvascular Angiogenesis model is constructed using GFP-Tagged human Retina Microvascular Endothelial cells. They are co-cultured with RFP-Tagged human supporting cells. GFP positive human retinal capillary like tubule formation can be monitored in real time under fluorescence microscope throughout the whole process of the experiment.

Advantages:

1) Cells used in the 3D model are all human cells; results obtained are more relevant to human situations rather than those data from animal models, i.e. CAM et al.
2) The whole angiogenesis process can be monitored (from cell inoculation to the end of experiment) therefore, more crucial information can be acquired at multiple time points from a single experiment.
3) No need to perform post-experimental staining for endothelial markers, this is particularly important, if those markers are changed in experimental conditions involved in the studies.

The 3D Human Retina Microvascular Angiogenesis model contains all of the materials necessary to perform 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 et al) can be monitored throughout the whole process under inverted fluorescence microscope.

Introduction:

Angiogenesis is a multistep process whereby new blood vessels develop from preexisting vasculature. Angiogenesis plays a key role in numerous physiological and pathological processes and understanding the mechanism of angiogenesis will therefore provide new approaches to the treatment of a wide range of pathologies. Angiogenesis is a complex process in which the following events are believed to play a critical role:

- Proteolytic degradation of the extracellular matrix
- Directed migration of endothelial cells
- Proliferation of endothelial cells
- Deposition of new extracellular matrix
- Formation of tubules and anastomosis of the newly formed vessels.

The 3D Human Retinal Microvascular Angiogenesis Kit series products from Alphabioregen 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. 3D Human Retinal Microvascular Angiogenesis Kit contains all of the materials necessary to perform multiple angiogenesis assays in a 24-well format. The kit is designed so 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 et al) can be monitored throughout the whole process under inverted fluorescence microscope.

Reagents and Materials Provided:

(1) 1 x vial of mixture of GFP-tagged Human Retina Microvascular ECs and RFP-tagged human supporting cells (-80°C or liquid N2)
(2) 1 x 24-well AlphaBio coated plate (Room temperature, for 2 months) (3) 1 x 500ml of Endo-Growth Medium (4°C)

Protocols: Day 1
1. Pre warm Endo-Growth Medium to 37ºC in a water bath
2. Accurately pipette 24ml Endo-Growth Medium into a 50ml Falcon tube
3. Rapidly thaw the vial of cryopreserved cells in a 37ºC water bath
4. Transfer all cells gently into 24ml pre warmed Endo-growth medium
5. Mix well the cells gently using a serological pipette
6. Add 1.0ml of cell suspension to each well of the pre coated 24-well plate.
7. Make sure the cells are evenly dispersed in the wells.
8. Place the plate in an incubator (37ºC, 5% CO2 and humidified

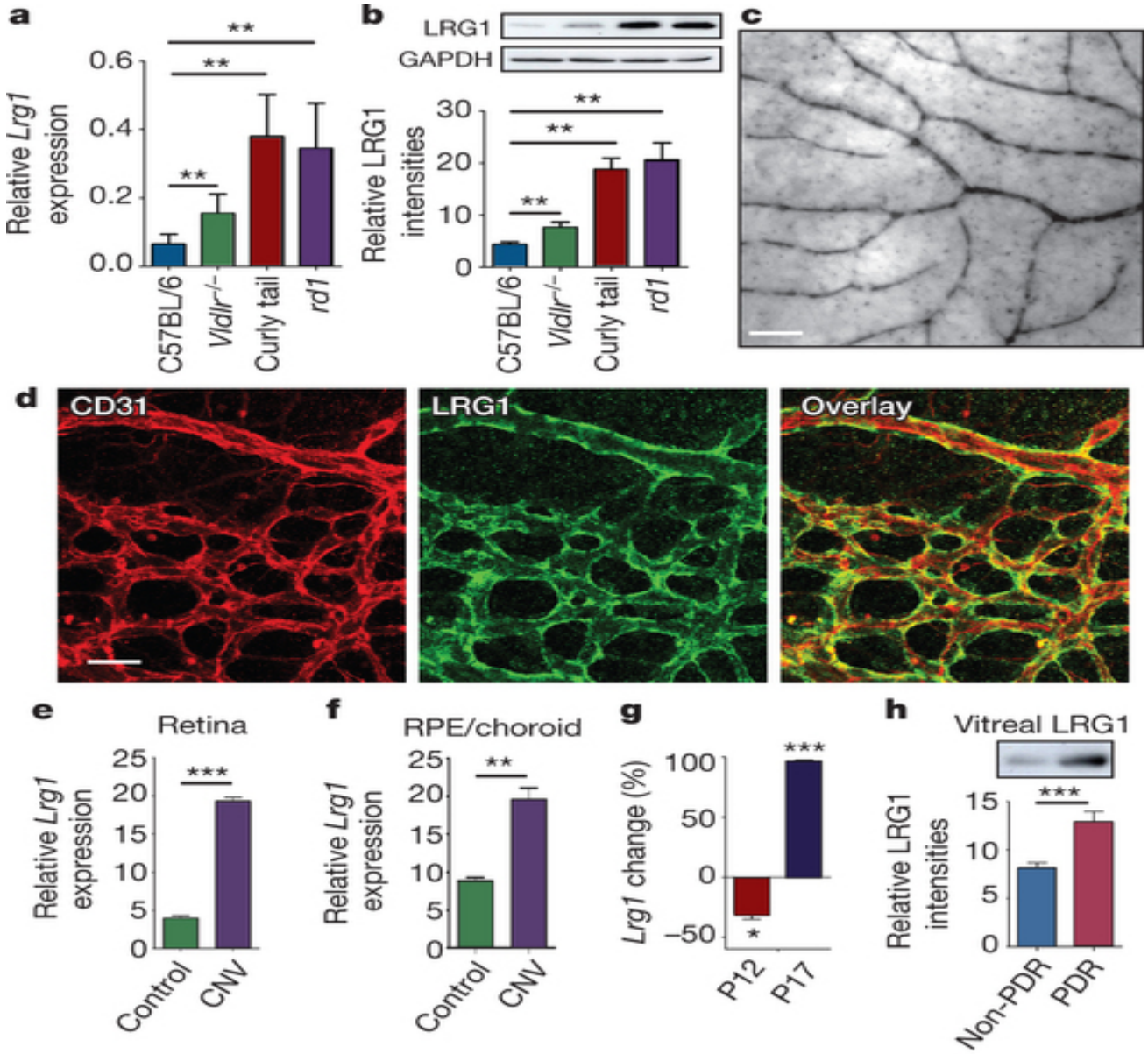
Day 2
9. Take the plate from the incubator and examine cells under inverted fluorescence microscopy (GFP positive Human Retina Microvascular ECs should sparsely and evenly distributed among RFP positive human mesenchymal supporting cells).

10. Wash the cells once with 2 ml of PBS

11. Add 2.0ml of fresh Endo-Growth medium (control) or Experimental media (Endo-Growth medium, plus pro- or anti-angiogenic regents according to customer's needs).

12. Place the plate back into the incubator. Day 4, 6, 8, 10, 12, and 14……

13. Replace the medium every 2 days until the end of the experiments.


a
Relative Lrg1 expression
0.6
0.4
0.2
0.0
**
**
**
C57BL/6
Vldlr−/−
Curly tail
rd1
b
LRG1
GAPDH
Relative LRG1 intensities
30
20
10
0
**
**
**
C57BL/6
Vldlr−/−
Curly tail
rd1
c
d
CD31
LRG1
Overlay
e
Retina
Relative Lrg1 expression
25
20
15
10
5
0
***
Control
CNV
f
RPE/choroid
Relative Lrg1 expression
25
20
15
10
5
0
**
Control
CNV
g
Lrg1 change (%)
100
50
0
−50
***
*
P12
P17
h
Vitreal LRG1
Relative LRG1 intensities
15
10
5
0
***
Non-PDR
PDR