11 Park Drive, Suite 12
Boston, MA 02215

In vitro Human Retina Microvascular Angiogenesis Tracing Kit
(24-well plate format)

Ordering Information

Name of Product: In vitro Human Retina Microvascular Angiogenesis Tracing Kit
Cat No: **cAP-16**

Introduction:

Angiogenesis is a multistep process whereby new blood vessels develop from pre-existing 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: (1) Proteolytic degradation of the extracellular matrix; (2) Directed migration of endothelial cells; (3) Proliferation of endothelial cells; (4) Deposition of new extracellular matrix; and (5) Formation of tubules and anastomosis of the newly formed vessels.

The human AngioTracing Kit series products from Angio-Proteomie incorporates a proprietary system in which GFP-tagged human endothelial cells from variable vascular beds are co-cultured with RFP-tagged human mesenchymal 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. These gradually join up (by 1 - 2 weeks) to form a network of anastomosing tubules which closely resembles the capillary bed found in vivo.

The Human Retina Microvascular Angiogenesis Tracing Kit (cAP-16) contains all of the materials necessary to perform multiple angiogenesis assays in a 24-well format. The kit 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.

Reagents and Materials Provided

(1) 1 x vial of mixture of GFP-tagged Human Retina Microvascular ECs and RFP-tagged human mesenchymal supporting cells (-80°C or liquid N2)
(2) 1 x 24-well Quick Coating Solution coated (cAP-01) plate (Room temperature, for 2 months)
(3) 1 x 500ml of Endo-Growth Medium (cAP-02) (4°C)

Contact & Ordering Information: Angio-Proteomie, 11 Park Drive, Suite 12, Boston, MA 02215, USA. Tel: 617-549-2665; Fax: (480) 247-4337, angioproteomie@gmail.com

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Protocols:

Day 1

1. Prewarm 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 precoated 24-well plate.
7. Make sure the cells are evenly dispersed in the wells.
8. Place the plate in an incubator (37ºC, 5% CO_2 and humidified).

Day 2

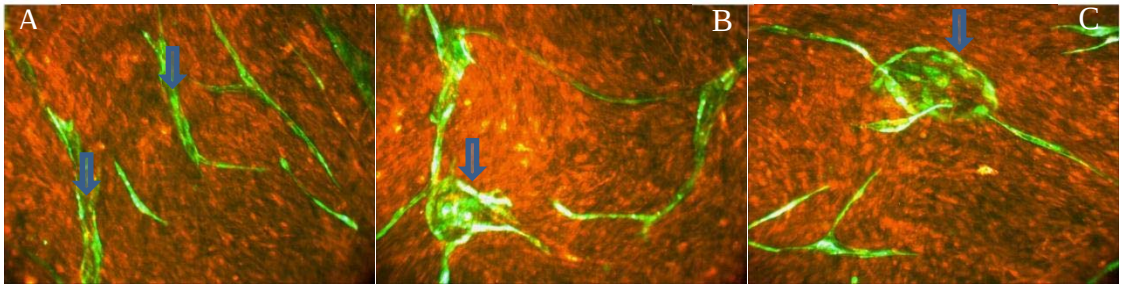
9. Take the plate from the incubator and examine cells under inverted fluorescence microscopy (GFP positive Human Retina Microvascular ECs should sparsely and evenly distributed among RFP positive human mesenchymal supporting cells).
10. Wash the cells one with 2 ml of PBS
11. Add 2.0ml of fresh Endo-Growth 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.

(NOTE: Other commonly used endothelial growth medium may also been used with our Human Retina Microvascular AngioTracing kit, but customer need optimize conditions before using those medium).

The images of endothelial tubes can be monitored and recorded at any time during the whole experimental process under inverted fluorescence microscope.



The capillary like tubules (Green) are shown in the above images after the cells were cultured with Endo-Growth Medium (cAP-02) for 12days. Tubules with lumens are indicated by arrows in A. Retinal aneurysm like structures are also observed and indicated by arrows in B and C. Human mesenchymal supporting cells (Red) are shown in the background.

This product is commercially produced for Research Use Only and under no circumstances that the product can be used for any human applications-10

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