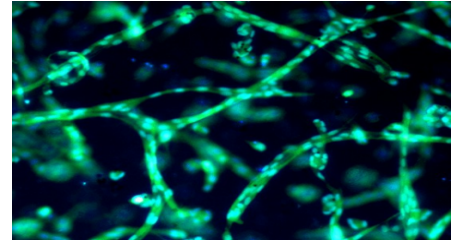


## 3D Human GFP-Tagged Lymphatic Model

<b>Product Name</b>	3D Human GFP-Tagged Lymphatic Model
<b>Catalog Number</b>	EP006
<b>Product Format</b>	6, 12, and 24 well
<b>Storage</b>	37°C

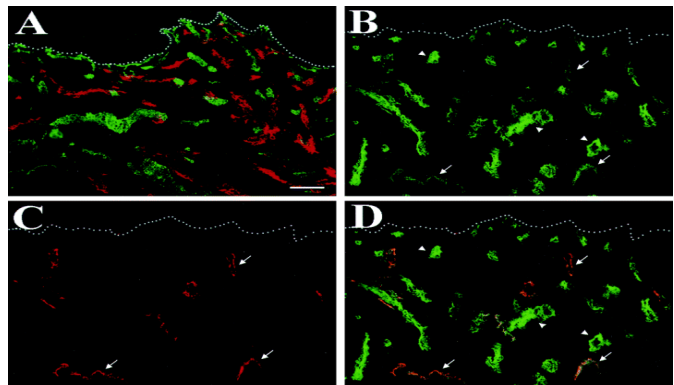
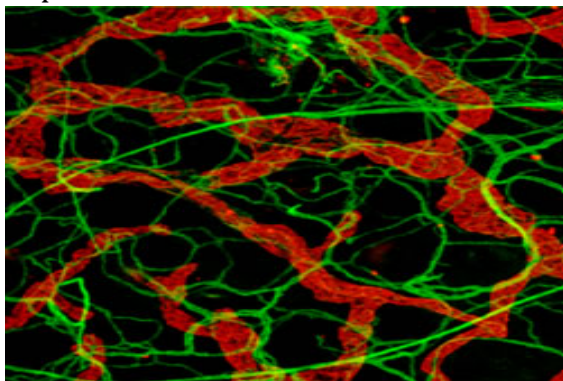


### GENERAL INFORMATION

Lymphangiogenesis is a multistep process whereby new blood vessels develop from pre-existing lymphatic vasculature. Lymphoangiogenesis 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. Similar to vascular angiogenesis, lymphangiogenesis is also a complex process in which the following events are believed to play a critical role:

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

Our 3D Human GFP-Tagged Lymphatic endothelial cells are co-cultured with RFP- Tagged human supporting cells. GFP positive lymphatic capillary like tubule formation can be monitored in real time under fluorescence microscope throughout the whole process of the experiment.



- 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. 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 GFP-Tagged lymphatic 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.

## Reagents and Materials Provided

1. 1 x vial of mixture of GFP-tagged Human lymphatic ECs and RFP-tagged human supporting cells (-80°C or liquid N<sub>2</sub>)
2. 1 x 24-well plate Coated with AlphaBioCoat Solution (Room temperature)
3. 1 x 500ml of Endo-Growth Medium(4°C)

### Day 1

1. Pre-warm Endo-Growth Medium to 37°C 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 37°C 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 (37°C, 5% CO<sub>2</sub>, and humidified).

### Day 2

9. Take the plate from the incubator and examine cells under inverted fluorescence microscopy
10. (GFP positive Human lymphatic ECs should sparsely and evenly distributed among RFP positive human supporting cells).
11. Wash the cells one with 2 ml of PBS

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

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.