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Angiogenesis (Tube Formation) Assay

rev. 5/19

(Catalog # K905-50; 50 assays; Store at -20°C)

I. Introduction:

Angiogenesis is the process of generating new blood vessels from the pre-existing vasculature. Angiogenesis is required for growth and development, wound healing, tissue granulation and formation of malignant tumors. The quick assessment of angiogenesis involves measurement of the ability of endothelial cells to form three-dimensional tube-like structures. BioVision's Angiogenesis (Tube Formation) Assay provides a robust method to determine angiogenesis (*in vitro*) in less than 18 hr. This assay kit provides a simple, easy to perform, semi-quantitative tool for assessing angiogenesis.

II. Application:

- Screening inhibitors and stimulators of angiogenesis
- Study of angiogenesis related signal transduction

III. Sample Type:

Small molecules or recombinant proteins

IV. Kit Contents:

Components	K905-50	Cap Code	Part Number
Extracellular Matrix Solution	2 x 1.25 ml	Red	K905-50-1
Wash Buffer	10 ml	NM	K905-50-2
Staining Dye Concentrate	25 µl	Green	K905-50-3
Inhibitor Control (Suramin)	1 vial	Amber	K905-50-4

V. User Supplied Reagents & Equipment:

- Endothelial cells (primary cells or immortalized cell line)
- Endothelial cell culture media
- Humidified cell culture incubator at 37°C with 5% CO_2
- Light and fluorescence microscope (with FITC filter set or equivalent)
- 96-well clear plate for cell culture

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C. Read the entire protocol before performing the assay. Open all the reagents under sterile conditions (*e.g.* laminar flow cell culture hood).

- **Extracellular Matrix Solution: For long-term (6 months) storage, we recommend aliquoting under sterile conditions to several tubes and storing at -20°C. Avoid freeze thaw cycles.** Always thaw slowly on ice or in a frost-free 4°C refrigerator. **Temperature above 4°C will rapidly gel the Extracellular Matrix Solution.** Thawing may take overnight at 4°C. The thawed matrix can be stored at 2-8°C for one week.
- **Wash Buffer:** Store at -20°C. Warm to 37°C before use.
- **Staining Dye Concentrate:** Store at -20°C.
- **Inhibitor Control (Suramin):** Reconstitute with 110 µl of dH_2O and vortex to yield a 10 mM stock solution. The 10 mM solution should be stored at -20°C, protected from light and is stable for 3 freeze/thaw cycles. The active concentration of Suramin will vary depending on the cell type. **We recommend using Suramin at a final concentration of 10-40 µM, a dose range that has been shown to exhibit strong anti-angiogenic effects without inducing overt cytotoxicity.**

VII. Tube Formation Assay Protocol:

1. **Cell Culture:** Grow endothelial cells in desired media up to ~90% confluency (37°C incubator containing 5% CO_2). Harvest cells under sterile conditions using basic cell culture techniques. Resuspend the cells in desired culture media containing 0.5-5% serum.
2. **Tube Formation:** Add 50 µl of thawed Extracellular Matrix Solution to each well of a pre-chilled (on ice) 96-well sterile cell culture plate. Make sure the gel spreads evenly on the surface of the well (rock or tap gently to spread). Incubate for 1 hr at 37°C to allow the solution to form a gel. Seed approximately 1-2 x 10^4 endothelial cells/well using 100 µl culture medium/well. For Inhibitor Control (Surmain) wells, seed the same number of cells/well in 100 µl culture medium containing desired concentration of Suramin. Add cells onto the solidified Extracellular Matrix gel (or No Extracellular Matrix gel control wells). Grow cells for 4-18 hr in a 37°C incubator containing 5% CO_2.

   Note: If desired, test compounds (i.e. stimulants or inhibitors of angiogenesis) other than the included Inhibitor Control (Suramin) may be added to designated test wells during the tube-formation incubation period. If an organic solvent is used to dissolve test compound, we recommend also performing a vehicle condition (with the same final concentration of solvent) in order to control for possible effects of the solvent.
3. **Data Analysis:** Carefully remove the medium using a pipette without disturbing the cells or the Extracellular Matrix gel. Gently wash the wells with 100 µl of Wash Buffer to remove serum. Remove the Wash Buffer carefully. Prepare 100 µl/well of Staining Dye working Solution by diluting Staining Dye Concentrate 1:200 (*e.g.* 5 µl of Staining Dye Concentrate in 995 µl of Wash Buffer) according to the number of wells. Add 100 µl of Staining Dye working solution to each well. Incubate for 30 min at 37°C. Examine the endothelial tube formation using light and fluorescence microscopy (FTIC/eGFP filter). We recommend acquiring several images per well.

Several options are available to analyze the pictures. For manual analysis, we recommend using image manipulation software e.g. Image J (free from NIH). For automated analysis, we recommend using the WimTube image analysis tool. It is based on tubule characteristics (*i.e.* number of tubules, number of junctions, tubule length, and number of loops).

Note:

Prepare Staining Dye working solution immediately before use. Staining Dye working solution is stable for 1 hr at 4°C.

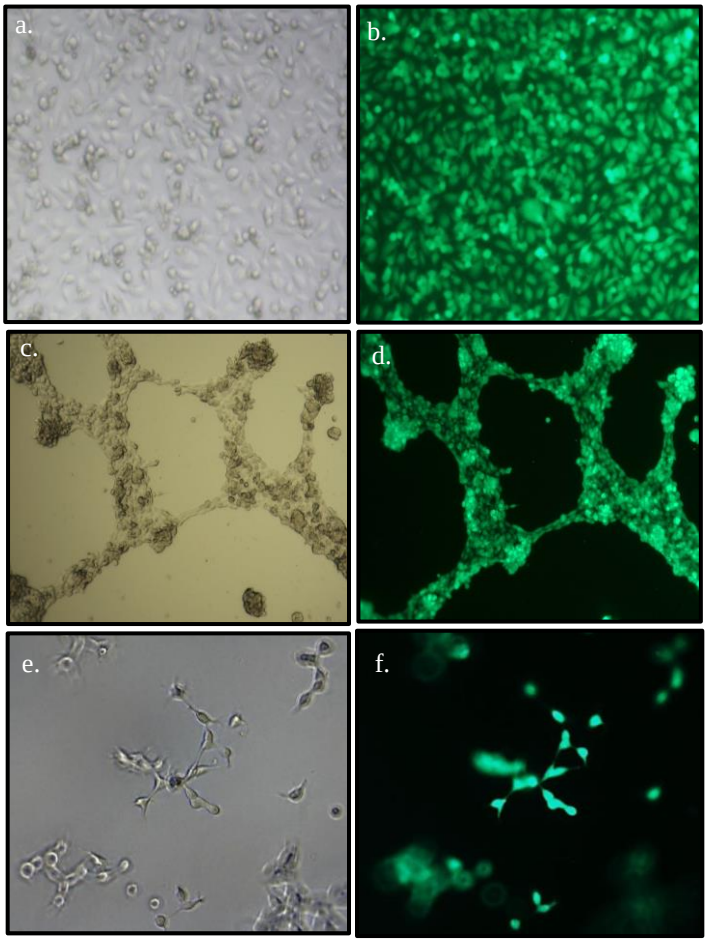

Figure: Endothelial Cell (EA.hy926 Cells) Tube Formation: Phase contrast (**a**, **c**, **e**) and fluorescent images (**b**, **d**, **f**) of endothelial cells in a tissue culture plate. (**a**, **b**) Endothelial cells grown without the Extracellular Matrix Gel, (**c**, **d**) Tube formation of endothelial cells grown on Extracellular Matrix gel. (**e**, **f**) endothelial cells grown on Extracellular Matrix gel treated with Suramin (10 µmol/L). Images were taken using Nikon TE2000 microscope.

VIII. Related Products:

Angiopoietin-1 (human) ELISA Kit (K7115-100)
Angiopoietin-2 (human) ELISA Kit (K7116-100)
Angiogenin (human) ELISA Kit (K4802-100)
Cholesterol/Cholesteryl Ester Quantitation Colorimetric/Fluorometric Kit (K603)
Cholesterol/Cholesteryl Ester Quantitation Colorimetric Kit II (K623)
HDL and LDL/VLDL Quantitation Colorimetric/Fluorometric Kit (K613)
CETP Activity Fluorometric Assay Kit (K601, K595)
P5091 (2277)
Vinblastine Sulfate (1959)
VisionBlue™ Quick Cell Viability Fluorometric Assay Kit (K303)
2-Methoxyestradiol (2166)
BIBF1120 (2167)
Bleomycin sulfate (2246)
Thiabendazole (2161)
NVP-BHG712 (2464)
P529 (2462)
Fumagillin (2368)

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