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ab204726 Angiogenesis Assay Kit (In Vitro)

View Angiogenesis Assay Kit (In Vitro) datasheet: www.abcam.com/ab204726

For the measurement of angiogenesis and the effect of inhibitors/ stimulators.

This product is for research use only and is not intended for diagnostic use.

Table of Contents

1. Overview

Tube Formation Assay (ab204726) provides a robust method to determine angiogenesis *in vitro* in less than 18 hours. This product provides a simple, easy to perform, qualitative tool for assessing angiogenesis.

Grow endothelial cells to ~90% confluence. Harvest and resuspend in media containing 0.5 – 5% serum.

Add cells (1 -1 x 10^4 cells per well) to Extracellular Matrix Gel. Add PTPase Inhibitor (Suramin) to Inhibitor Control wells. Omit gel from No Extracellular Matrix control wells. Add test compounds to desired wells.

Incubate cells for 4-18 hours in a 37°C incubator containing 5% CO_2.

Add Dye and incubate at 37°C for 30 minutes.

Analyze cells by light and fluorescence microscopy.

2. Materials Supplied and Storage

Store kit at -20°C in the dark immediately on receipt and check below for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.

Avoid repeated freeze-thaws of reagents.

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Extracellular Matrix Solution	2 x 1.25 mL	-20°C	-20°C
Elution Buffer I	10 mL	-20°C	-20°C
Staining Dye Concentrate (Sterile)	25 µL	-20°C	-20°C
Inhibitor (Suramin)	1 vial	-20°C	-20°C

PLEASE NOTE: Inhibitor (Suramin) was previously labelled as PTPase Inhibitor and Inhibitor Control (Suramin), and Staining Dye Concentrate (Sterile) as Staining Dye Concentrate, and Elution Buffer I as Wash Buffer III. The composition has not changed.

3. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Human umbilical vein endothelial cells (HUVEC) or other endothelial cells – primary cells or cell line can be used
- Appropriate endothelial cell culture media with serum
- Incubator at 37°C with 5% CO_2
- Inverted fluorescence microscope equipped with filter for Ex/Em = 490/540 nm (green fluorescence)
- General tissue culture supplies
- 96-well clear plate for cell culture
- Pipettes and pipette tips

4. General guidelines, precautions, and troubleshooting

Please observe safe laboratory practice and consult the safety datasheet.

For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide:
www.abcam.com/assaykitguidelines

For typical data produced using the assay, please see the assay kit datasheet on our website.

5. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

ΔNote: Open all the reagents under sterile conditions (e.g. cell culture hood).

5.1 Extracellular Matrix Solution:

Ready to use as supplied. Always thaw slowly on ice or in a frost free 4°C refrigerator. The thawed Extracellular Matrix Solution may be stored at 4°C for one week.

ΔNote: Once thawed on ice, the component may be aliquoted and stored at -20C for long term storage, or stored at +4C if used within 1 week. The component must be kept chilled; temperature increases above +4C will result in permanent polymerization of the basement membrane matrix gel.

5.2 Elution Buffer I:

Ready to use as supplied. Equilibrate to 37°C before use. Store at -20°C.

5.3 Staining Dye Concentrate (Sterile):

Ready to use as supplied. Aliquot Dye so that you have enough volume to perform the desired number of assays. Store at -20°C.

5.4 Inhibitor (Suramin)

Reconstitute with 110 µl of dH_2O and vortex to yield a 10 mM stock solution. The 10mM solution should be stored at -20°C, protected from light and is stable for 3 freeze/thaw cycles. The active concentration of PTPase Inhibitor (Suramin) will vary depending on the cell type. We recommend using PTPase Inhibitor (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.

6. Assay Procedure

- Assay all controls and samples in duplicate.
- Each cell line should be evaluated on an individual basis to determine the optimal cell density to perform the experiment.
- Treat cells with test compounds for desired period of time to induce response.
- Appropriate incubation time depends on the individual cell type and cell concentration used. Optimize the incubation time for each experiment.

6.1 Cell culture:

1. Grow endothelial cells in desired media up to ~90% confluency (37°C incubator containing 5% CO2).
2. Harvest cells under sterile conditions using basic cell culture techniques. Resuspend the cells in the desired culture media containing 0.5 - 5% serum.

6.2 Tube formation:

1. Add 50 µL of thawed Extracellular Matrix Solution to each well of a pre-chilled (on ice) black-walled, clear-bottomed, cell-culture-compatible 96 well sterile plate. Make sure the gel spreads evenly on the surface of the well (rock or tap gently to spread). Incubate for 1 hour at 37°C to allow the solution to form a gel.

   Background Control well = no Extracellular Matrix Solution.

2. Use approximately 1-2 x 10^4 endothelial cells/well for a black-walled, clear-bottomed, cell-culture-compatible 96 well sterile plate using 100 µl media/well. Add cells onto the solidified Extracellular Matrix gel or control wells (No Extracellular Matrix gel or Extracellular Matrix wells with PTPase Inhibitor (Suramin)). Add angiogenesis factors/regulators to the desired wells.
3. Incubate cells for 4-18 hours in a 37°C incubator containing 5% CO2.

6.3 Tube Staining:

1. Carefully remove the medium using a pipette without disturbing the cells or the Extracellular Matrix gel.

2. Gently wash the wells with 100 µL of Elution Buffer I to remove serum. Remove the Elution Buffer I carefully.
3. Prepare 100 µL/well of Staining Dye Working Solution by diluting Staining Dye Concentrate1:200 (e.g. 5 µL of Staining Dye Concentrate in 995 µL of Elution Buffer I) according to the number of wells. Add 100 µl of Staining Dye Working Solution to each well.
4. Incubate for 30 minutes at 37°C.
5. Examine the endothelial tube formation using light and fluorescence microscopy (green filter).

7. Data Analysis

We recommend acquiring several images per well. To ensure objective and quantitative analysis, we recommend using imaging software.

Manual analysis:

For manual analysis, if you do not have a specific software installed in your microscope, you can download ImageJ, an open source image processing designed for scientific multidimensional images by the National Institute of Health (NIH).

Automated analysis:

For automated analysis, we recommend using Wimasis WimTube Solution. This tool is based on tubule characteristics such as number of tubules, number of junctions, tubule length, and number of loops.

8. Typical Data

Data provided **for demonstration purposes only.**

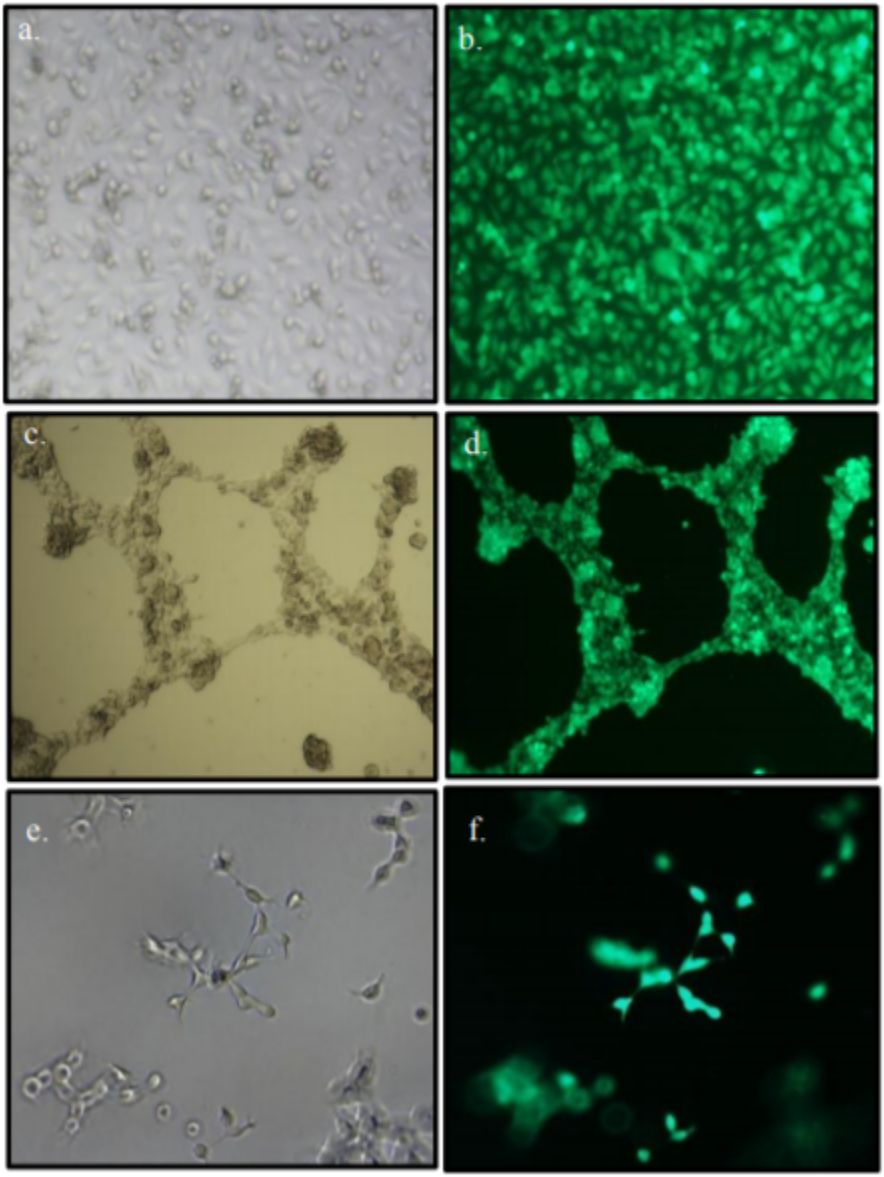

Figure 1. Endothelial Cell (EA.hy926 Cells) Tube Formation: Phase contrast (a, c, e) and fluorescent images (b, d, f) of endothelial cells in a tissue culture plate. (a, b) Endothelial cells grown without the Extracellular Matrix Gel, (c, d) Tube formation of endothelial cells grown on Extracellular Matrix gel. (e, f) endothelial cells grown on Extracellular Matrix gel treated with PTPase Inhibitor (Suramin) (10 µmol/L). Images were taken using Nikon TE2000 microscope.

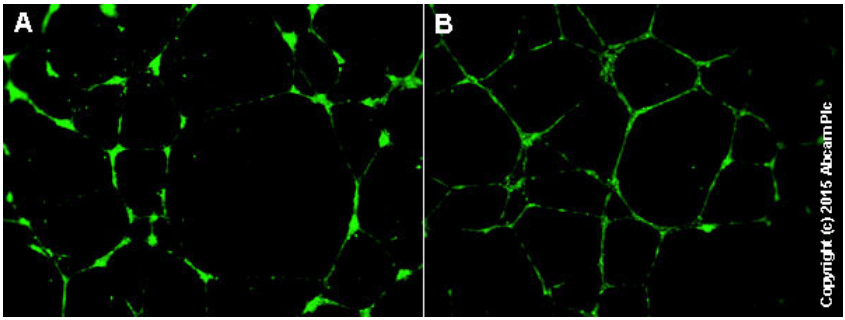

Figure 2. HUVEC morphogenesis on Extracellular Matrix Gel. Cells (2×10^4) were plated per 1 cm^2 well precoated with Extracellular Matrix Gel and grown for 18 hours (A) in the specific medium alone (positive control) or containing (B) PMA 10 µmol/L.

9. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)