ANGIOGENESIS PRODUCTS

Tube Formation

Angiogenesis

Endothelial

Cell Adhesion

3-D Culture

Cell Invasion / Cell Migration
CANCER CELL ASSAYS

The behavior of cancer cells has both intrigued and plagued scientists for years. As a provider of tools for cancer research, Trevigen has developed and optimized extracellular matrix based assay formats to assess the behavior of cancer cells. Keeping the researcher in mind, we emphasized sensitivity, accuracy, and ease of use. The culmination of this development work is a series of products and methods designed to study cancer progression at the cellular level. These include assays that measure the critical cellular functions of adhesion, proliferation, migration, and invasion, as well as 3-D assays that may be used to assess cellular differentiation, morphology, angiogenic potential, and molecular composition of cells within their physiological microenvironment.

IN VIVO ANGIOGENESIS ASSESSMENT

Trevigen's DIVAA™ (Directed In Vivo Angiogenesis Assay) is the first in vivo assay that provides quantitative and reproducible angiogenesis assessment results.

The DIVAA kits provide implant grade silicone cylinders closed at one end, called angioreactors. The angioreactors are filled with 20 µl of Trevigen's Basement Membrane Extract (BME) premixed with or without angiogenesis modulating factors. These angioreactors are then implanted subcutaneously in the dorsal flanks of nude mice. If filled with angiogenic factors, vascular endothelial cells migrate into, and proliferate in the BME to form vessels in the angioreactor. As early as nine days post-implantation, there are enough cells to determine an effective dose response to angiogenic factors. The sleek design of the angioreactor provides a standardized platform for reproducible and quantifiable in vivo angiogenesis assessment assays.

**FIGURE 1**

**FIGURE 2**

The basement membrane extract provides the correct microenvironment for vessel formation in response to chemoattractants; FGF-2 and an FGF-2/VEGF mix are provided as a control.

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<td>DIVAA Inhibition Kit</td>
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<td>AngioRack™</td>
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**DIVAA Starter Kit** - The Directed In Vivo Angiogenesis Assay (DIVAA) Starter Kit was designed to introduce the technology and give the user practical experience assessing angiogenesis. It contains 48 angioreactors, enough growth factor to induce angiogenesis in all 48 angioreactors, and an AngioRack™ designed to hold the angioreactors during the course of assay setup.

**DIVAA Activation Kit** - The Directed In Vivo Angiogenesis Assay (DIVAA) Activation Kit was designed for assessing angiogenesis activation. It contains 48 angioreactors and enough growth factor for eight positive controls. DIVAA has been employed in evaluating the enhancement of angiogenesis associated with adrenomedullin and CD97.

**DIVAA Inhibition Assay** - The Directed In Vivo Angiogenesis Assay (DIVAA) Inhibition Kit was designed for assessing angiogenesis inhibition. It contains 48 angioreactors and enough growth factor to induce angiogenesis in all 48 angioreactors. DIVAA has been utilized in demonstrating the inhibition of angiogenesis in matrix metalloprotease (MMP)-2-deficient mice by TIMP-2.

**DIVAA AngioRack** - Also available as a separate product, the autoclavable and reusable AngioRack is designed to hold 48 sterile silicone angioreactors. The rack is designed for convenient filling of reactors prior to implantation.
The tube formation kit contains all the components necessary to perform the assay, including Growth Factor Reduced Basement Membrane Extract (BME), Calcein AM, Cell Staining Solution, and Sulforaphane. Cultrex Reduced Growth Factor (RGF) BME provides the appropriate microenvironment for endothelial cells to align, migrate and form three-dimensional capillary-like structures in vitro. Sulforaphane [1-isothiocyanato-(4R)-methylsulfinyl)-butane], found in broccoli and other cruciferous vegetables, is a naturally occurring cancer chemopreventive agent, and is provided as a control inhibitor of in vitro endothelial cell tube formation on Cultrex BME. Calcein AM is provided for rapid and accurate measurement of cell viability and/or cytotoxicity, and real-time kinetic analysis of tube formation.

Human Umbilical Vein Endothelial Cells (HUVEC) were cultured on gelled RGF BME for four hours at 37°C and 5% CO₂ in Endothelial Basal Medium (no serum or angiogenic factors) (panel A), Endothelial Growth Medium-2 (with serum, supplements and growth factors) in the absence (panel B) or presence of 15 µM Sulforaphane (panel C); and then labeled with 2 µM Calcein AM. Images were taken using 484 nm excitation / 520 nm emission filter on a fluorescent microscope equipped with 10X objective.

ENDOTHELIAL CELL INVASION

The Endothelial Cell Invasion kit offers a flexible, standardized, high-throughput format for quantitating the degree to which endothelial cells penetrate an in vitro BME barrier in response to chemoattractants and/or inhibiting compounds. This assay employs a simplified Boyden chamber-like design with an 8 micron polyethylene terephthalate (PET) membrane. Sulforaphane, a naturally occurring cancer chemopreventive agent, is provided as a control for inhibition of in vitro endothelial cell migration/invasion on Cultrex BME. Ports within the migration/invasion chamber (top) allow access to the assay chamber (bottom) without dismantling the device. This design is easier to use, prevents contamination, and is adaptable for robotic high-throughput systems. The assay chamber may be directly analyzed in a 96 well plate reader, eliminating transfer steps that introduce additional variability to the assay. The permissiveness of the BME matrix may also be optimized to fit each experiment by adjusting the coating concentration.
Maintenance Treatment with Bevacizumab Prolongs Survival in an In vivo Ovarian Cancer Model

Seiji Mabuchi, Yoshito Terai, Kenichiro Morishige, Akiko Tanabe-Kimura, Hiroshi Sasaki, Masanori Kanemura, Satoshi Tsunetoh, Yoshimichi Tanaka, Masahiro Sakata, Robert A. Burger, Tadashi Kimura, and Masahide Ohmichi


Hypoxia stimulates pancreatic stellate cells to induce fibrosis and angiogenesis in pancreatic cancer

Atsushi Masamune, Kazuhiro Kikuta, Takashi Watanabe, Kennichi Satoh, Morihisa Hirota, and Tooru Shimossegawa


Deletion of Yin Yang 1 Protein in Osteosarcoma Cells on Cell Invasion and CXCR4/Angiogenesis and Metastasis

Filomena de Nigris, Raffaele Rossiello, Concetta Schiano, Claudio Arra, Sharon Williams-Ignarro, Antonio Barbieri, Alessandro Lanza, Antonio Balestrieri, Maria Teresa Giuliano, Louis J. Ignarro, and Claudio Napoli


Defective angiogenesis, endothelial migration, proliferation, and MAPK signaling in Rap1b-deficient mice

Magdalena Chrzanowska-Wodnicka, Anna E. Kraus, Daniel Gale, Gilbert C. White, II, and Jillian VanSluys


Genetic Evidence for a Noncanonical Function of Seryl-tRNA Synthetase in Vascular Development

Wiebke Herzog, Katja Müller, Jan Huisken, and Didier Y.R. Stainier


Tissue-engineered endothelial and epithelial implants differentially and synergistically regulate airway repair

Brett G. Zani, Koji Kojima, Charles A. Vacanti, and Elazer R. Edelman


TECHNICAL PRESENTATIONS

DIVAA™

1. **What is DIVAA™?**

The Directed In Vivo Angiogenesis Assay (DIVAA) invented and developed at the National Institutes of Health by William Stetler-Stevenson, is the first in vivo system for the study of angiogenesis that provides quantitative and reproducible results. Angioreactors (silicone cylinders closed at one end) containing 20 µl of basement membrane extract with/without angiogenic-modulating factors are implanted subcutaneously in the dorsal flank of nude mice. With the onset of angiogenesis, vascular endothelial cells proceed to grow into the basement membrane extract and form vessels in the angioreactor. As early as nine days post-implantation, there are enough cells to determine an effective dose response to angiogenic modulating factors. (Cat #3450)

2. **How does DIVAA compare to the plug assay?**

The DIVAA (Cat #3450) angioreactor prevents assay errors due to re-adsorption of the basement membrane extract by the mouse. The assay also has lower dose requirements for angiogenic modulating factors compared to the plug assay, and multiple data points can be generated in one mouse.

3. **Why does DIVAA contain a FGF/VEGF mixture to promote angiogenesis?**

While FGF-2 and VEGF have both been demonstrated to promote angiogenesis in DIVAA, the FGF/VEGF mixture provides a synergistic effect allowing a drastic increase in response using lower growth factor concentration. (Cat #3450)

4. **What is the difference between the FITC Lectin and FITC Dextran in the DIVAA protocol?**

FITC-Lectin specifically binds to endothelial cells, so it counts the total number of endothelial cells contained within the angioreactor. FITC-Dextran does not bind endothelial cells. Instead, it is dispersed within the blood of the mouse, being evenly distributed within the blood vessels, so it quantitates the total volume of blood contained within the vessels within the angioreactor. Both procedures have demonstrated equivalent results empirically.

5. **Can multiple conditions be employed in a single mouse?**

Using multiple conditions in the same mouse results in higher variability, so it is not recommended. It is hypothesized that angiogenesis modulating factors may be entering the blood stream and affecting angiogenesis in other locations within the animal.

6. **What is the Tube Formation Assay and what are its advantages?**

The Tube Formation Assay is based on the ability of endothelial cells to form three-dimensional capillary-like tubular structures when cultured on a hydrogel of reconstituted basement membrane extract (BME). The Tube Formation Assay is rapid, inexpensive and quantifiable. It can be used to identify potentially angiogenic and anti-angiogenic factors; to determine endothelial cell phenotype, and to study pathways and mechanisms involved in angiogenesis. It can be performed in a high throughput mode to screen for a large number of compounds. Therefore, the Tube Formation Assay is the most widely used in vitro angiogenesis assay.

7. **What cell types can be used in Tube Formation Assay?**

Tube Formation Assay is specific for endothelial cells: either primary cells or immortalized endothelial cell lines. Only endothelial cells form capillary-like structures with a lumen inside, other cell types form other structures.

8. **What are the variables associated with the Tube Formation Assay?**

The major variables associated with tube formation are composition of the BME hydrogel, thickness of hydrogel, cell density, composition of angiogenic factors in the assay media, and assay period.

9. **Which BME should I use for the Tube Formation Assay?**

Reduced Growth Factor BME (RGF BME) is generally used for testing compounds that promote angiogenesis because formation of capillary-like structures (tubes) is significantly less, compared to complete basement membrane matrix (non-reduced BME). Trevigen's Tube Formation Kit includes a qualified production lot of RGF BME that exhibits reduced background tube formation in the absence of angiogenic factors.

10. **How do I reduce spontaneous formation of tubular structures on BME gels in the absence of angiogenic factors?**

Primary endothelial cells, such as Human Umbilical Vein Endothelial Cells (HUVECs) form capillary-like structures in the absence of added angiogenic factors less often than immortalized endothelial cells. Titrate the number of cells and find optimal conditions for your specific cell line. When endothelial cells make fully-formed capillary structures in response to angiogenic activators, but not in their absence, you may proceed. Generally, reducing the number of cells per cm² of gelled BME, results in less background or spontaneous tube formation.
WHAT CUSTOMERS SAY

Martin Jechlinger, PhD
Varmus Laboratory
Cancer Biology and Genetics Program, SKI
Memorial Sloan-Kettering Cancer Center

“We have used Trevigen’s Cultrex® reduced growth factor matrix in combination with rat Collagen I to grow primary mammary cells in 3-D cell culture conditions. The reliable quality of these matrices was essential to grow primary cells into polarized acini and to subsequently study effects of oncogene induction and de-induction.”

Samir Alhasan, PhD
Asterand

“We have used, to our satisfaction, Trevigen’s 3-D Culture Matrix BME to grow human cell lines in 3-D cell culture conditions.”

RELATED PRODUCTS

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<td>Cultrex® 24 Well Collagen I Cell Invasion Assay</td>
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<td>Cultrex® Fibronectin 96 Well Cell Adhesion Assay</td>
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We are pleased to announce the immediate availability of PathClear™ Basement Membrane Extract in the Cultrex® product line. The PathClear™ designation means that in addition to standard sterility, endotoxin and MAP testing, the BME is tested by PCR and is clear of a battery of 31 pathogens*, including LDEV.

Each lot is rigorously qualified in biological performance assays. Available in reduced growth factor form, with and without phenol red, PathClear™ BME is expected to be of special interest for in vivo murine research work and other work requiring BME free from viruses, bacteria and mycoplasma.

Trevigen, Inc. develops, manufactures & markets an extensive line of Cultrex® brand products for the promotion of cell growth, cellular differentiation and for the study of cancer cell behavior.

For details, contact your local office of AMS Biotechnology at info@amsbio.com or visit our website at www.amsbio.com

* PCR Tested clear of Mycoplasma spp., LDEV, SENDAI, MHV, PVM, MVM, MPV(1,2,3), NOROVIRUS, REO3, EDIM, ECTROMELIA, LCMV, K, MTV, POLYOMA, HANTAAN, MAD(1,2), MCMV, KILHAM'S, TOOLAN'S, PARVO, RCMV, CORONA, RMV, SEOUL, SIALODACRYOADENITIS, TMEV, TMELV

MAP Tested clear of Mycoplasma pulmonis, E. cuniculi, MHV, TMEV, MMV, PVM, MVM, HANTAAN, SENDAI, MTV, MCMV, ECTROMELIA, LCMV, LDEV, POLYOMA, K Virus, REO(1,3), MPV, EDIM, MAV(1,2)

**Comparitive Table for Matrigel™ Users**

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<th>BD Product Description</th>
<th>Trevigen Cat. #</th>
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<td>Basement Membrane Matrix, phenol red free, 10 ml</td>
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<td>Cultrex BME without phenol red, PathClear™, 5ml</td>
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<td>354248</td>
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<td>3444-005-02</td>
<td>Cultrex High Protein Concentration BME, PathClear™, 5ml</td>
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Trevigen, Inc. is a rapidly growing biotechnology company focused on the development of products and technology for cancer research, emphasizing apoptosis, DNA damage and repair, and cancer cell function and behavior. Working with AMS Biotechnology since 1992 Trevigen has been a long-standing provider of quality reagents and kits for researchers investigating programmed cell death and DNA damage and repair. A logical extension of this focus on cancer research has been the recent development of assays for cancer cell function and behavior including angiogenesis, cell invasion and tumor formation. Through AMS Biotechnology in Europe Trevigen offers contract screening services employing CometAssay™, PARP and in vitro angiogenesis assays.

Immunology – Genomics – Proteomics – Electrophoresis – Cell Culture

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