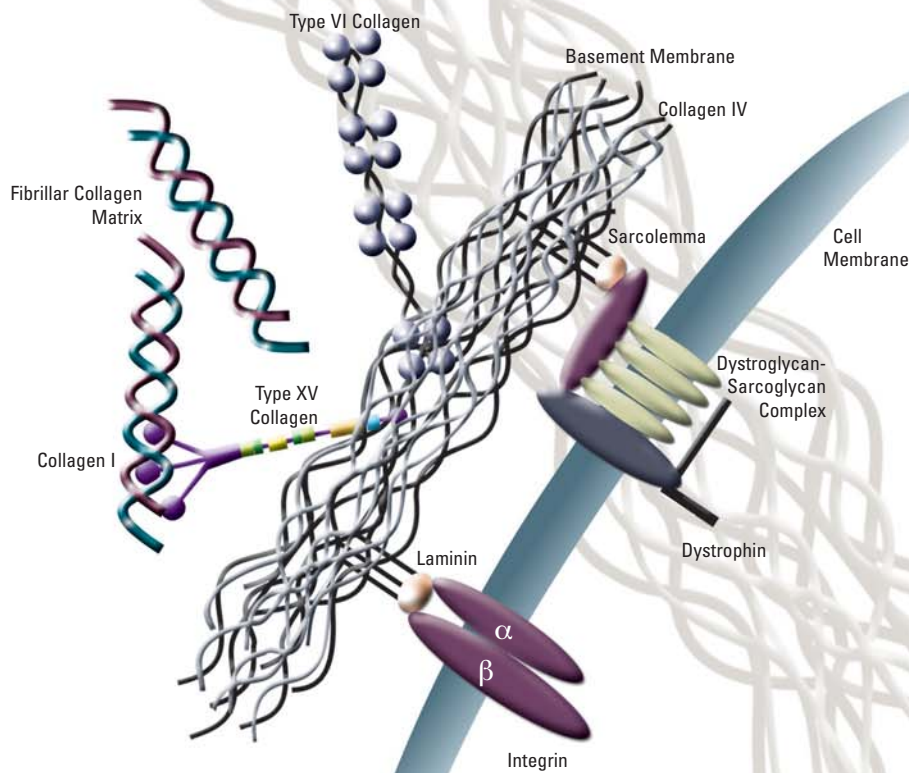


CULTREX® BASEMENT MEMBRANE EXTRACT and SPECIALTY PROTEINS



TREVIGEN®

BME PRODUCTS

amsbio
info@amsbio.com

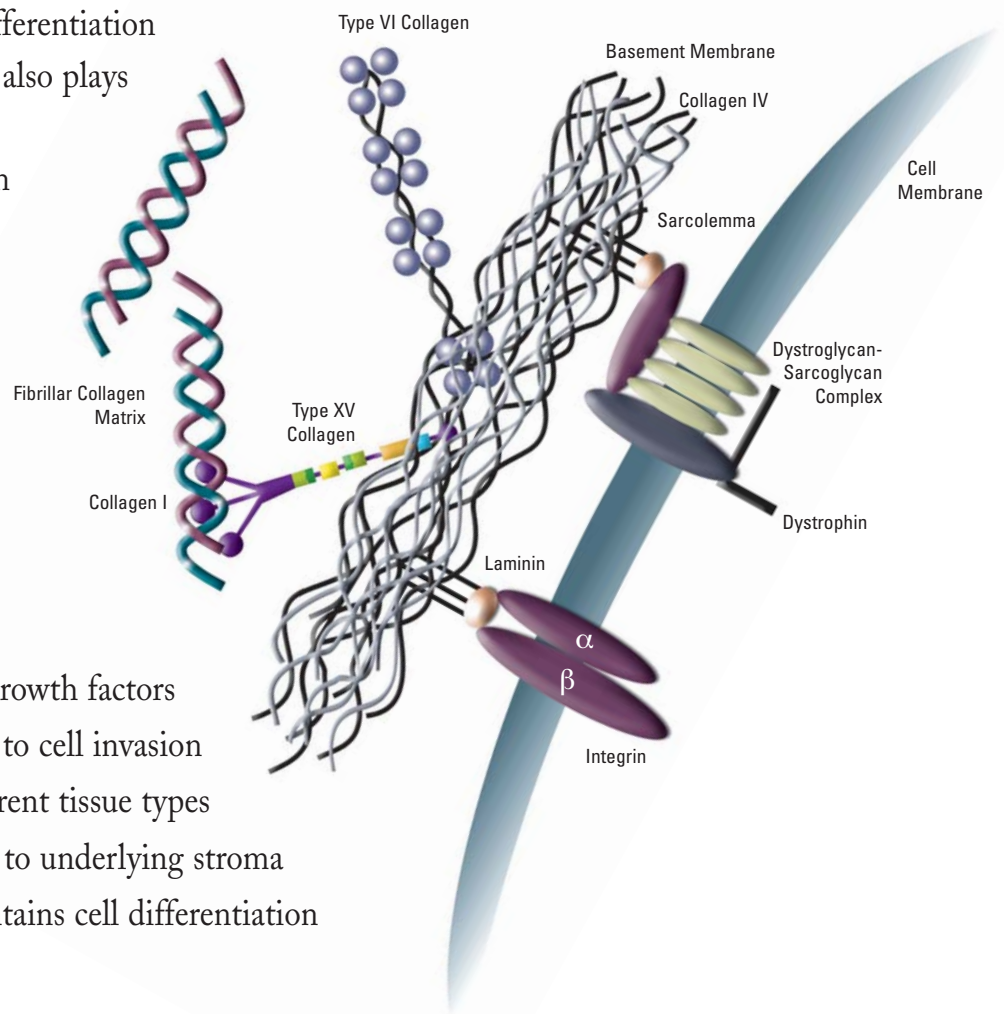
BASEMENT MEMBRANES

Basement membranes are continuous sheets of specialized extracellular matrix that are found at the base of all lumen-lining epithelial and endothelial cells. They are composed of specialized basement membrane proteins, including collagen IV (the primary structural element of the basement membrane), laminin I, heparin sulfate proteoglycan, and entactin. The primary function of the basement membrane is to anchor down the epithelium to its loose connective tissue underneath. This is achieved by cell-matrix adhesions through cell adhesion molecules (CAMs). The basement membrane acts as a mechanical barrier, preventing malignant cells from invading the deeper tissues. The basement membrane is also essential for angiogenesis.

Basement membrane proteins have been found to accelerate differentiation of endothelial cells. It also plays an important role in glomerular filtration in the kidney.

Functions of Basement Membranes:

- Filtration
- Storage depot for growth factors
- Mechanical barrier to cell invasion
- Separates two different tissue types
- Anchor epithelium to underlying stroma
- Promotes and maintains cell differentiation



IN VITRO DIFFERENTIATION

CELLS/EXPLANT	RESPONSE	REFERENCE/ PROTOCOLS
Cell lines		
Prostate*	Acinar formation, glands	Webber, M.M. et al. 1997. Acinar differentiation by non-malignant immortalized human prostatic epithelial cells and its loss by malignant cells. <i>Carcinogenesis</i> 18:1225-1231.
Salivary*	Acinar formation, amylase production	Royce, L. et al. 1993. Human neoplastic submandibular intercalated duct cells express and acinar phenotype when cultured on a basement membrane matrix. <i>Differentiation</i> . 52:247-255.
Mammary epithelial*	Duct and lumina formation, increased casein	Seely, K.A. & Aggeler, J. 1991. Modulation of milk protein synthesis through alteration of the cytoskeleton in mouse mammary epithelial cells cultured on a reconstituted basement membrane. <i>J. Cell Physiol.</i> 146: 117-130.
MDCK* Cells	Polarized cyst	Rahinkkala, M. et al 2001. Effects of SRC Kinnse and TGF beta I on the differentiation and morphogenesis of MDCK cells grown in three-dimensional collagen and matrigel environments, <i>J. Pathol</i> , 195: 391-400.
Pancreas Cells	Acinar differentiation	Arias, A.E. & Bendayan, M. 1993. Differentiation of pancreatic acinar cells into duct-like cells Invitro. <i>Lab Invest.</i> 69:518-530.
Schwann cells*	Differentiation	
Intestinal cells*	Differentiation	Sanderson, T.R. et al. 1996. Human fetal enterocytes in vitro : modulation of phenotype by extracellular matrix <i>Proc. Natl. Acad Sci.</i> 1996 93:-7717-7722.
Bone cells	Canaliculi formation	Vukiceric, S. et al. 1990. Differentiation of Canalicular cell processes in bone cells by basement membrane matrix components: regulation by discrete domains of laminin. <i>Cell.</i> 63 : 437-445.
Blastocyst stem cells	Immature glandular & tubular structures	Philip, D. et al. 2005 Complex extracellular matrices promote tissue- specific stem cell differentiation, <i>Stem Cells.</i> 23:288-296.
Primary cells		
Sertoli Cells	Columnar epithelium	Papadopoulos, V. & Dym , M. 1994. Sertoli Cell differentiation on the basement membrane is mediated by C-fos proto oncogene <i>Pro Natl. Acad. Sci. USA</i> 91:7027-7031.
Hepatocytes*	Morphology maintained, albumen production	Friedman, S.L. et al. Maintenance of differentiated phenotype of cultured rat lipocytes by basement membrane matrix. <i>J. Biol. Chem.</i> 264:10756-10762.
Chondrocytes	Cartilage formation	Bradham, D.M. et al. 1995. Mesenchymal cell chondrogenesis is stimulated by basement membrane matrix and inhibited by age associated factors. <i>Matrix Biol.</i> 14:561-571.
Endothelial cells*	Capillary tubes with lumen	Morale, D.E et al. 1995. Estrogen promotes angiogenic activity in human umbilical vein endothelial cells in vitro and in a murine model. <i>Circulation</i> 91:755-763.
Endometrial cells	Columnar epithelium, glands	Strunck, E. et al. 2001. Expression of 1-3- phosphoserine phosphatase is regulated by reconstituted basement membrane <i>Biochem. Biophys, Res Common.</i> 281:747-753.
Oviduct epithelium	Tubes with ciliated cells	Joshi, M.S. 1991 Growth and Differentiation of the cultured secretory cells of the cow oviduct on reconstituted basement membrane. <i>J.Exp. Zool.</i> 260:229-238.
Murine Prostate Stem Cell	Form spheroids and prostate tubular structures	Xin, L. et al. 2007. Self –renewal and multilineage differentiation in vitro from murine prostate stem cells. <i>Stem Cells.</i> 25:2760-2769.
Tissue explants	Outgrowth	Newby, D. 2005. Villous explant culture : Characterization and evaluation of a model to study trophoblast invasion. <i>Hypertens. Pregnancy.</i> 2005 24:75-91.
Neural crest	Outgrowth	Bilozur, M.E. & Hay, E.D 1988. Neural crest migration in 3D Extracellular matrix utilizes laminin, fibronectin, or collagen, <i>Dev Biol.</i> 125:19-33.
Dorsal root ganglia explants	Outgrowth with myelin production	Carey, D.J. et al. 1986. . Schwann cell myelination: induction by exogenous basement membrane-like extracellular matrix. <i>J. Cell Biol.</i> 1986 102:2254-2263.
Immature follicles	Hair growth	Harvlickova, B. et al. 2004. Towards optimization of an organotypic Assay system that imitates hair follicle like epithelial mesenchymal interactions <i>Br. J Dermazol.</i> 2004 151:753-765.
Aortic rings	Vessel outgrowth	Malinoa, K.M. et al. 1999. Identification of laminin alpha 1 and beta 1 chain peptides action for endothelial cell adhesion, tube formation and aortic sprouting, <i>FASEB.</i> 1999. 13:53-62 .
Ookinetes (zygote)	Sporogonic development of malaria parasite	Warburg, A. & Miller, L.H. 1992. Sporogonic development of malaria parasite in vitro. <i>Science.</i> 255:448-450.

*denotes activity with both primary cells and cell lines.

BIOLOGICAL ACTIVITY

Cell differentiation in vitro	Allows for the development of model systems for studying gene expression and activity, ex. Production factories for proteins
Tumor growth in vivo	Increased incidence and 'take' of tumor cells and biopsy material in subcutaneous or orthotopic sites
Transplant survival in vivo	Increased survival of tissue transplants in the brain
Repair of epithelial tissue in vivo	Repair of intestinal ulcers with a basement membrane patch
Fat growth in vivo	Subcutaneous implants induce fat formation
Assays for angiogenesis	Capillary-like tube assay in vitro; Sprouting vessels form vessel segments ex vivo; Subcutaneous implants in vivo
Assays of tumor cell malignancy	Morphology on the matrix in vitro; Invasion through the matrix in vitro
Tissue engineering	Tissue replacement with stem cells in vivo; Tissue replacement with cells that have formed organoids in vitro and then implanted in vivo
Stem cell growth	Supports growth and differentiation of stem cells

PRODUCTS

Basement Membrane Extracts

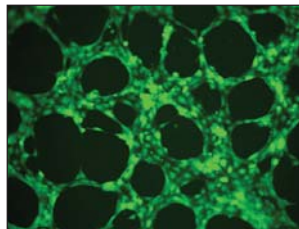
Multipurpose Cultrex® BME (Basement Membrane Extract)

Multipurpose BME is available in concentrations ranging from 12-17 mg/ml and is qualified for angiogenesis, tumorigenicity assays, migration, growth, differentiation, and neurite outgrowth assays. It is available either reduced or non-reduced in growth factors and either with phenol red or without. All four standard BME types and our specialty BME matrices are available in trial sizes for your testing convenience. BME lots can be reserved for your research requirements. Each lot of BME has passed sterility testing following USP XXIV chapter 71 sterility test and is negative for mycoplasma by PCR.

Description	Size	Catalog No.
Cultrex® BME with Phenol Red	5 ml	3430-005-01
Cultrex® BME with Phenol Red, Reduced Growth Factor	5 ml	3431-005-01
Cultrex® BME, No Phenol Red	5 ml	3432-005-01
Cultrex® BME, No Phenol Red, Reduced Growth Factor	5 ml	3433-005-01

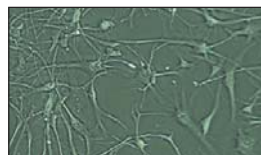
Cultrex® BME PathClear™

Cultrex® BME PathClear™ is sterile for bacterial and fungal growth according to USP XXIV chapter 71 sterility test. It has also been tested and cleared for mycoplasma as well as 31 other pathogens and viruses including LDEV (Lactate Dehydrogenase Elevating Virus).



Human Blood Microvascular Endothelial Cells (HBMVEC) were pre-incubated for 30 minutes with 2µM CalceinAM and then cultured on gelled PathClear™ RGF BME for four hours at 37°C and 5% CO2 in Endothelial Growth Medium-2MV. Image was taken at 10X magnification.

Description	Size	Catalog No.
Cultrex® BME with Phenol Red, PathClear™	5 ml	3430-005-02
Cultrex® BME with Phenol Red, Reduced Growth Factor PathClear™	5 ml	3431-005-02
Cultrex® BME, No Phenol Red, PathClear™	5 ml	3432-005-02
Cultrex® BME, No Phenol Red, Reduced Growth Factor PathClear™	5 ml	3433-005-02

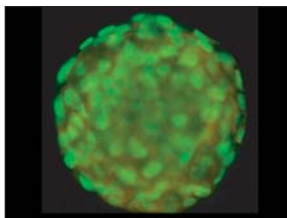


Dopaminergic neuronal cells grown on PathClear™ BME. Image courtesy of: Thiru V. Gopal Conexpress, Inc. Gaithersburg, MD

PRODUCTS

Cultrex® 3-D Culture Matrix™

Trevigen provides three different matrices capable of forming 3-D substrates for cell culture and differentiation. The 3-D Culture Matrix™ BME is our reduced growth factor BME qualified, lot-to-lot, to promote differentiation of human epithelial cell lines derived from mammary gland (MCF-10A) and human prostate (PC-3) into acinar structures. This matrix provides the foundation for cells to grow in three dimensions allowing for the formation of structures in vitro and is available at a consistent concentration. The 3-D Culture Matrix™ Laminin I and the 3-D Culture Matrix™ Collagen I may be used as a gel on which to grow cells or a media additive alone or in concert with other basement membrane components to study cellular growth and differentiation in three dimensions in vitro. 3-D Culture Matrix™ Laminin I is available at a consistent concentration of 6 mg/ml (by absorbance and extinction coefficient) and the 3-D Culture Matrix™ Collagen I at 5 mg/ml (Sircol Assay).



Description	Size	Catalog No.
Cultrex® 3-D BME, No Phenol Red, Reduced Growth Factor	15 ml	3445-048-01
Cultrex® 3-D Laminin I	5 ml	3446-005-01
Cultrex® 3-D Collagen I - Rat Tail	100 mg	3447-020-01

3-D Culture of MCF-10A cells on 3-D Culture Matrix™ Collagen I: in Assay Media with 2% BME stained with SYBR® Green and propidium iodide.*

Cultrex® HC20+™ BME PathClear™

Cultrex® HC20+™ BME is available at a concentration of 20 mg/ml or greater for those requiring a high concentration BME for in vivo angiogenesis assays and tumorigenicity assays. Cultrex® BME PathClear™ has been tested and cleared of 31 pathogens and viruses including LDEV (Lactate Dehydrogenase Elevating Virus) making it ideal for in vivo work. Patent pending.

Description	Size	Catalog No.
Cultrex® High Protein BME (HC20+™) PathClear™	5 ml	3444-005-02

CITATIONS

Recent Citations using Trevigen BME Products –
Product descriptions and catalog numbers on pages 3 and 4

Interleukin-6 is a potent growth factor for ER—positive human breast cancer

A. Kate Sasser, Nicholas J. Sullivan, Adam W. Studebaker, Lindsay F. Hendey, Amy E. Axel, and Brett M. Hall
FASEB J, Jun 2007; 10.1096/fj.07-8832com.

Identification of proangiogenic TIE2-expressing monocytes (TEMs) in human peripheral blood and cancer

Mary Anna Venneri, Michele De Palma, Maurilio Ponzoni, Ferdinando Pucci, Cristina Scielzo, Erika Zonari, Roberta Mazzieri, Claudio Doglioni, and Luigi Naldini
Blood, Jun 2007; 109: 5276 – 5285.

Human herpes virus 8 acute infection of endothelial cells induces monocyte chemoattractant protein 1-dependent capillary-like structure formation: role of the IKK/NF- κ B pathway

Elisabetta Caselli, Simona Fiorentini, Carla Amici, Dario Di Luca, Arnaldo Caruso, and M. Gabriella Santoro
Blood, Apr 2007; 109: 2718 – 2726.

Semaphorin 4D provides a link between axon guidance processes and tumor-induced angiogenesis

John R. Basile, Rogerio M. Castilho, Vanessa P. Williams, and J. Silvio Gutkind
PNAS, Jun 2006; 103: 9017 – 9022.

PRODUCTS

Trevigen offers a range of specialty proteins including Mouse Laminin I, Mouse Collagen IV, Rat Collagen I, Bovine Collagen I, Bovine Fibronectin and Bovine Vitronectin.

Specialty Proteins

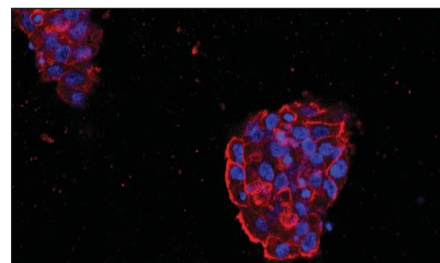
Collagen I is the major structural component of extracellular matrices found in connective tissue and internal organs. Collagen I is most prevalent in the dermis, tendons, and bone. It is a 300 kDa molecule composed of two alpha1(I) chains and one alpha2(I) chain that spontaneously forms a triple helix scaffold when at a neutral pH and 37°C. Collagen I promotes cell attachment, growth, differentiation, migration, and tissue morphogenesis during development.

Collagen IV is the primary collagen found in the extracellular basement membrane matrices, separating a variety of epithelial and endothelial cells from the underlying tissues. Trevigen's mouse collagen IV is purified from the EHS sarcoma, where it comprises up to 10% of the total tumor mass. It can be used as a filter, for coating on tissue culture surfaces, to promote cell attachment and proliferation and to study its effects on cell behavior. It is also used in cell invasion assays.

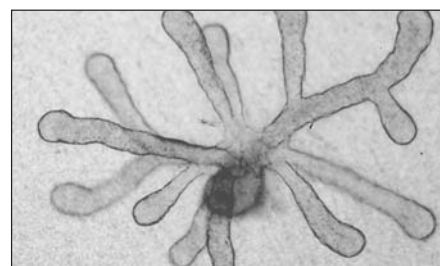
Laminin I is an extracellular matrix protein which contains a number of functional domains. Trevigen's highly purified Laminin I, from the EHS sarcoma, increases cell adhesion, migration, growth, and differentiation, including neurite outgrowth. It is composed of a 1b1g1chain with a total Mr of 800,000 and is used for coating culture dishes and invasion assays. At high concentrations, it will gel.

Fibronectin is a 440 kDa, soluble disulfide-linked dimer composed of two 220 kDa peptide chains. It is an extracellular matrix protein that is found abundantly in blood, connective tissues, and provisional matrices associated with malignant transformation of migratory cells. Fibronectin functions in both cell-cell and cell-matrix interactions. Fibronectin functions either as a general cell adhesion molecule or as a modulator in binding between cell surfaces and the extracellular matrix by means of a central cell-binding domain, RGD (Arg-Gly-Asp).

Vitronectin is an extracellular soluble disulfide-linked dimer composed of a 75 kDa and a 65 kDa peptide chain with a molecular weight of 140 kDa. Vitronectin is a major plasma glycoprotein that promotes cellular adhesion and spreading, inhibits the membrane-damaging effect of the terminal cytolytic complement pathway, and binds to several serpin (serine protease inhibitors). Vitronectin can be used for coating tissue culture surfaces to promote cell adhesion or as an additive for serum-free medium.



MCF10A cells grown in collagen I gel and stained with hoechst and anti-b-catenin antibody to visualize cell boundaries. Image courtesy of J. Partanen & J. Klefstrom, University of Helsinki. Partanen, J.I., Mäkelä, T.P. and Klefstrom, J. 2007. Suppression of oncogenic properties of c-Myc by LKB1-controlled epithelial organization. PNAS, 104: 14694 - 14699.



Primary embryonic submandibular epithelium cultured in laminin-1 gel with FGF10 (200 ng/ml). The epithelia are cultured for either 24 or 48 hours in serum-free media and undergo branching morphogenesis in culture. Image courtesy of Matthew P. Hoffman. Patel, V.N., Knox, S.M., Likar, K.M., Lathrop, C.A., Hossain, R., Eftekhari, S., Elkins, M., Vlodyasky, I., Whitelock, J.M. and Hoffman, M.P. Heparanase cleavage of heparin sulfate modulates FGF10 function during submandibular gland branching morphogenesis. Development, In Press.

Description	Size	Catalog No.
Cultrex® Bovine Collagen I	50 mg	3442-050-01
Cultrex® Bovine Fibronectin	1 mg	3416-001-01
Cultrex® Bovine Vitronectin	50 mg	3417-001-01

Description	Size	Catalog No.
Cultrex® Rat Collagen I	100 mg	3440-100-01
Cultrex® Mouse Collagen IV	1 mg	3410-010-01
Cultrex® Mouse Laminin I	1 mg	3400-010-01

Accessory Products

Poly-L-Lysine, a highly positively-charged amino acid chain, is commonly used as a coating agent to promote cell adhesion in culture. This solution is provided ready to use at 0.01% and contains polymers in the 70,000-150,000 kDa range.

Description	Size	Catalog No.
Cultrex® Poly-L-Lysine	100 ml	3438-100-01

CITATIONS

Recent Citations using Trevigen Specialty Proteins and Accessory Products –
Product descriptions and catalog numbers on page 5

Retinoschisin Is a Peripheral Membrane Protein with Affinity for Anionic Phospholipids and Affected by Divalent Cations

Mouse Collagen IV

Camasamudram Vijayasathy, Yuichiro Takada, Yong Zeng, Ronald A. Bush, and Paul A. Sieving
Invest. Ophthalmol. Vis. Sci., Mar 2007; 48: 991 – 1000.

Suppression of oncogenic properties of c-Myc by LKB1-controlled epithelial organization

Rat Collagen I

Johanna I. Partanen, Anni I. Nieminen, Tomi P. Mäkelä, and Juha Klefstrom
PNAS, Sep 2007; 104: 14694 – 14699.

Heparanase cleavage of perlecan heparan sulfate modulates FGF10 activity during ex vivo submandibular gland branching morphogenesis

Laminin I

Vaishali N. Patel, Sarah M. Knox, Karen M. Likar, Colin A. Lathrop, Rydhwana Hossain, Siavash Eftekhari, John M. Whitelock, Michael Elkin, Israel Vlodaysky, and Matthew P. Hoffman
Development, Dec 2007; 134: 4177 – 4186.

A Cleavable Propeptide Influences Toxoplasma Infection by Facilitating the Trafficking and Secretion of the TgMIC2–M2AP Invasion Complex

Poly-L-Lysine

Jill M. Harper, My-Hang Huynh, Isabelle Coppens, Fabiola Parussini, Silvia Moreno, and Vern B. Carruthers
Mol. Biol. Cell, Oct 2006; 17: 4551 – 4563.

WHAT CUSTOMERS SAY

Hynda K. Kleinman
Guest, NIH

"We have used Cultrex® in all of our in vitro (endothelial cell tube formation, stem cell differentiation), ex vivo (aortic explant outgrowth, salivary gland organ differentiation), and in vivo assays (subcutaneous angiogenesis, tumor growth promotion) where it has worked well with highly reproducible findings. The fast delivery and availability was highly appreciated by me and my staff."

Brett M. Hall, Ph.D.
Assistant Professor of Pediatrics
The Ohio State University School of Medicine
Investigator, Center for Childhood Cancer
Columbus Children's Research Institute

"After comparing head-to-head Trevigen's Cultrex® BME and BD Biosciences Matrigel™ BME, Trevigen's Cultrex® equaled or exceeded BD's Matrigel™ BME product in our 3D Tumor Growth Assay (TGA). Cultrex® BME has a lower retail cost, higher average BME concentration, and superior lot-to-lot consistency with respect to tumor cell growth in the 3D TGA (Sasser, et al. 2007 Cancer Letters). Thank you for making an excellent product at an affordable cost."

Henry Lopez
President/CSO
MuriGenics, Inc.

"The product is of the highest quality, I'm particularly impressed with the quality assurance program (PathClear™ BME) you have in place. We look forward to continued use of your products."

RELATED PRODUCTS

Researchers who purchased BME products also purchased these...

Antibodies to Basement Membrane Proteins

Rat Collagen I Polyclonal Antibody

The rat collagen I polyclonal antibody can be used to demonstrate Collagen I synthesis by cells in culture, to localize Collagen I in tissues and in stroma and in fibrotic diseases, and to distinguish fibroblastic cell lineages from epithelial lineages. This antibody is reactive in ELISA, can be used for Western blots, and in Immunofluorescence for frozen and paraffin embedded tissue sections.

Description	Size	Catalog No.
Rat Collagen I Polyclonal Antibody	100 µl	3440-PC-100
Mouse Laminin I Polyclonal Antibody	100 µl	3400-PC-100
Bovine Fibronectin Polyclonal Antibody	100 µl	3416-PC-100
Bovine Vitronectin Polyclonal Antibody	100 µl	3417-PC-100

Mouse Laminin I Polyclonal Antibody

The mouse laminin I polyclonal antibody can be used to demonstrate laminin production by cells in culture, to define basement membrane localization in tissues and around blood vessels, nerves, and fat cells. It can also be used to distinguish epithelial from fibroblastic cells in tissues by Immunofluorescence.

Bovine Fibronectin Polyclonal Antibody

The bovine fibronectin polyclonal antibody can be used to demonstrate fibronectin synthesis by cells in culture and to localize fibronectin in tissues and tissue stroma. It can be used in ELISA and in the location of fibronectin in frozen and formalin fixed paraffin sections by immunofluorescence.

Bovine Vitronectin Polyclonal Antibody

The bovine vitronectin polyclonal antibody can be used in ELISA and for localizing vitronectin in frozen and formalin fixed paraffin embedded tissues by immunofluorescence.

Directed In Vivo Angiogenesis Assays

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.

Description	Size	Catalog No.
DIVAA™ Starter Kit	48 Tests	3450-048-SK
DIVAA™ Activation Kit	48 Tests	3450-048-K
DIVAA™ Inhibition Kit	48 Tests	3450-048-IK
AngioRack™	1 Rack	3450-048-09

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 Kit

The Directed In Vivo Angiogenesis Assay (DIVAA™) Inhibition Kit was designed for assessing angiogenesis inhibition. 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™ AngioRack™

Available as a separate product, the autoclavable, reusable AngioRack™ is precision machined of Teflon® to hold 48 sterile silicone angioreactors in the laminar flow hood while filling and preparing for implantation.

Cultrex® 3-D Culture Assays

Cultrex® 3-D Culture Cell Proliferation Assays

The Cultrex® 3-D Culture Cell Proliferation Assays are the first standardized kits engineered for assessing the effects of compounds or genetic alteration on cell proliferation in 3-D Culture. The assays are available with Cultrex® Basement Membrane Extract, Laminin I, or Collagen I; or it may be purchased without matrix for customers to evaluate their own 3-D culture materials.

Description	Size	Catalog No.
3-D Culture Cell Proliferation Assay Core Kit	96 Tests	3445-096-CK
3-D Culture BME Cell Proliferation Assay Kit	96 Tests	3445-096-K
3-D Culture Laminin I Cell Proliferation Assay Kit	96 Tests	3446-096-K
3-D Culture Collagen I Cell Proliferation Assay Kit	96 Tests	3447-096-K

Cultrex® 3-D Culture Cell Harvesting Kit

The Cultrex® 3-D Culture Cell Harvesting Kit is designed for optimal isolation of cells from 3-D Culture BME or Laminin I for subsequent biochemical analysis without degrading the proteins on the cell surface.

Description	Size	Catalog No.
3-D Culture Cell Harvesting Kit (human/mouse)	20 Tests	3448-020-K

FAQS

1. How should Basement Membrane Extract (BME) be stored and handled?

BME should be stored at or below -20°C in a manual defrost freezer. Preparation of working aliquots is recommended. BME should be thawed overnight on ice at 4°C (refrigeration). Long term storage at 4°C is not recommended due to temperature fluctuations. Freeze/thaw cycles and gel-liquid phase transitions can compromise product integrity. BME gels at 8°C therefore it must always be handled on ice. All materials should be chilled.

2. Can BME be diluted?

Yes, dilute BME in tissue culture media at physiological pH at 4°C . BME will form a gel when diluted to 10 mg/ml; however, further dilutions may require optimization.

3. How does Cultrex® BME promote cell differentiation?

All epithelial and endothelial cells are in contact with a basement membrane matrix on at least one of their surfaces. By providing them with their natural matrix in vitro, as a substrate for the cells that provides biological cues, the cells can assume a more physiological morphology (i.e. correct shape) and begin expression of cell lineage specific proteins. Two-dimensional plastic substrates, in combination with serum-containing media, cause cells to flatten, proliferate and de-differentiate.

FAQS

4. How does Cultrex® BME promote tumor growth?

Tumor cells and fragments of biopsy specimens grow well in vivo when implanted with Cultrex® BME. Typically less than 5% of biopsy specimens will grow when implanted directly but in our experience better than 95% of the tested specimens grew. The HC20+™ in vivo specifically promotes growth due to biological signals coming from the matrix components (i.e. laminin, collagen IV, etc) and the growth and angiogenic factors present in the matrix. When the proteases from tumor cells degrade the Cultrex® BME, the bioactive fragments of the matrix components and growth factors act directly on the tumor cells and also recruit nearby vessels to begin angiogenesis.

5. What kinds of cells will differentiate on Cultrex® BME?

Many different types of primary and established epithelial and endothelial cells have been found to differentiate on Cultrex® BME. Below are some examples. Investigators should test their cells for responses of interest.

Cell line	Response	Reference	Protocol
Primary Sertoli cells	Columnar epithelium when cultured on top of gelled BME. Highly differentiated cords when cultured inside of gelled BME	Hadley, M.A. Byers, S.W. Suarez-Quian, C.A. Kleinman, H.K., and Dym, M. 1985. Extracellular matrix regulates Sertoli cell differentiation, testicular cord formation, and germ cell development in vitro. <i>J. Cell Biol</i> 1985, 101:1511-1522	
Endothelial cells (primary and established)	Capillary like structures	Lawley T.J., Kubota Y. Induction of morphologic differentiation of endothelial cells in culture. <i>J. Invest. Dermatol.</i> 1989; 93: 59-61.	Tube Assay*
Breast epithelial	Form acini that produce milk	Li, M.L., Aggeler, J., Farson, D.A., Hatier, C., Hassell, J. and Bissell, M.J. 1987. Influence of a Reconstituted Basement Membrane and Its Components on Casein Gene Expression and Secretion in Mouse Mammary Epithelial Cells. <i>PNAS</i> 84: 136 - 140.	3-D Culture Assay*
Primary hepatocytes	Differentiated morphology and albumen production	Schuetz, E.G., Li, D., Omiecinski, C.J., Muller-Eberhard, U., Kleinman, H.K., Elswick, B. and Guzelian, P.S. 1988. Regulation of gene expression in adult rat hepatocytes cultured on a basement membrane matrix. <i>J Cell Physiol.</i> 134(3): 309-23.	3-D Culture Assay*
Stem cells	Multiple types of epithelial structures	Philp, D., Chen, S.S., Fitzgerald, W., Orenstein, J., Margolis, L., and Kleinman, H.K. 2005. Complex Extracellular Matrices Promote Tissue-Specific Stem Cell Differentiation. <i>Stem Cells.</i> 23: 288 - 296.	
Pancreatic acinar cells	Acinar formation	Arias, A.E. and Bendayan, M. 1993. Differentiation of pancreatic acinar cells into duct-like cells in vitro. <i>Lab Invest</i> 69(5): 518-30.	3-D Culture Assay*
Salivary gland cells	Acinar formation	M.P. Hoffman, M.C. Kibbey, J.J. Letterio, and H.K. Kleinman. 1996. Role of laminin-1 and TGF-beta 3 in acinar differentiation of a human submandibular gland cell line (HSG). <i>J. Cell Sci.</i> 109: 2013 - 2021.	3-D Culture Assay*
Lung type II cells	Columnar morphology and surfactant production	Rannels, S.R., Fisher, C.S., Heuser, L.J., and Rannels, D.E. 1987. Culture of type II pneumocytes on a type II cell-derived fibronectin-rich matrix. <i>Am J Physiol Cell Physiol.</i> 253: C759 - C765.	

FAQS

6. What types of explants can be grown on Cultrex® BME?

Various tissues have been placed on Cultrex® BME with good survival and cellular outgrowth. Below are some examples. (Investigators should test for additional responses with other types of tissues and let us know their results.)

Tissue	Response	Reference	Protocol
Salivary gland rudiment	Maturation with multiple acini	Steinberg, Z., Myers, C., Heim, V.M., Lathrop, C.A., Rebutini, I.T., Stewart, J.S., Larsen, M. and Hoffman, M.P. 2005. FGFR2b signaling regulates ex vivo submandibular gland epithelial cell proliferation and branching morphogenesis. <i>Development</i> . 132: 1223 - 1234.	3-D Culture Assay*
Aorta or blood vessel	Vessel outgrowth	Malinda, K.M., Nomizu, M., Chung, M., Delgado, M., Kuratomi, Y., Yamada, Y., Kleinman, H.K. and Ponce, M.L. 1999. Identification of laminin 1 and B1 chain peptides active for endothelial cell adhesion, tube formation, and aortic sprouting. <i>FASEB J</i> . Jan 1999; 13: 53-62.	Aortic Ring*
Immature hair follicle	Maturation of hair follicle	Link, R.E., Paus, R., Stenn, K.S., Kuklinska, E., and Moellmann, G., 1990. Epithelial growth by rat vibrissae follicles in vitro requires mesenchymal contact via native extracellular matrix. <i>J Invest Dermatol</i> . 95(2): 202-207.	
Chick spinal ganglia	Neurite outgrowth with myelination	Carey, D.J., Todd, M.S. and Rafferty, C.M. 1986. Schwann cell myelination: induction by exogenous basement membrane-like extracellular matrix <i>J. Cell Biol</i> . 102: 2254 -2263.	

7. What kinds of tumor cells/biopsy specimens grow in vivo?

All cell lines and tumor biopsy specimens (usually cut into small fragments) have been found to grow in vivo when implanted with Cultrex® BME. These include melanoma, intestinal, prostate, breast, lung, renal and liver cancers as well as even 3T3 cells.

8. How will non-tumorigenic cells/tissues grow or differentiate when implanted in vivo in Cultrex® BME?

Non transformed cells mixed with Cultrex® BME and implanted in vivo have been found to continue to survive and remain differentiated but generally do not grow. No normal tissues have been found to transform under these conditions. For example, Sertoli cells survive at least a week and retain their cord-like structures. We again encourage investigators to try different cell types and let us know their results.

9. Which basement membrane protein is best for studying invasion?

For investigating general cell invasion, BME or collagen I coated inserts can be used to look at invasion through connective tissue; this would give the most physiologically significant result for screening compounds or genes that inhibit or promote invasion. For researching specific aspects or mechanisms of cell invasion, the laminin I, collagen IV, and the collagen I kits provide purified proteins to study these specific interactions. The key difference is overall physiological significance vs. specific mechanism of action.

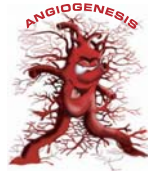
*Protocols: The protocols for 3D assays, Tube Formation, Aortic Ring Assay, Cell Invasion Assay and tumorigenicity assays can be found online at www.trevigen.com/cultrex.php

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