R&D SYSTEMS
a bio-techne brand

TREVIGEN®

3-D Matrices & Assay Kits

3-D Assay Kits & 3-D Matrices:

- Organoids
- Spheroids
- Embryoid Bodies
- Acinar Structures
- *In Vitro* Tumor Co-Culture

Organoid and 3-D Cell Culture Matrices

Organoid and three dimensional (3-D) cell culture systems are emerging as pivotal models for regenerative medicine, drug discovery, toxicology, and cancer research. Organoids are generated from pluripotent stem cells or organ progenitor cells that have differentiated and self-organized into a formation that resembles the structure and function of an intact mammalian organ. The *in vitro* generation of organoids requires an extracellular matrix that provides a growth scaffold with optimal tensile strength and molecular composition, including laminin, collagen IV, entactin, and heparin sulfate proteoglycan. R&D Systems has partnered with Trevigen® to offer a range of Cultrex® Basement Membrane Extracts that are ideal for generating organoid and 3-D cultures from pluripotent stem cells and cancer stem cells.

3-D KITS & MATRICES

Recent studies indicate that the composition of the extracellular environment influences cellular responses to apoptosis inducing agents implicating a role for extracellular proteins in influencing both toxicity and drug resistance. As a result, this environment must be mimicked during the course of cell-based studies to provide the most accurate translation to animal models.

PRODUCT NAME	CATALOG #	SIZE
Cultrex® 3-D Culture Matrix™ Reduced Growth Factor Basement Membrane Extract, PathClear®	3445-001-01	1 ml
	3445-005-01	5 ml
	3445-010-01	2 X 5 ml
Cultrex® Organoid Qualified Basement Membrane Extract (Type 2), PathClear®	3532-001-02	1 ml
	3532-005-02	5 ml
	3532-010-02	2 x 5 ml
Cultrex® Organoid Qualified, Reduced Growth Factor Basement Membrane Extract (Type 2), PathClear®	3533-001-02	1 ml
	3533-005-02	5 ml
	3533-010-02	2 x 5 ml
Cultrex® 3-D Culture Matrix Laminin I	3446-005-01	30 mg
Cultrex® 3-D Culture Matrix Rat Collagen I	3447-020-01	20 ml
Cultrex® 3-D Spheroid Cell Invasion Assay	3500-096-K	96 samples
Cultrex® 3-D Spheroid Fluorometric Proliferation/Viability Assay	3510-096-K	96 samples
Cultrex® 3-D Spheroid Colorimetric Proliferation/Viability Assay	3511-096-K	96 samples
Calcein-AM Cell Viability Assay Kit	4892-010-K	1000 wells

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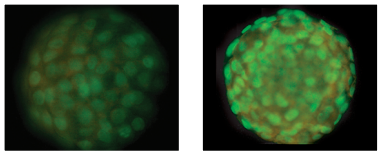
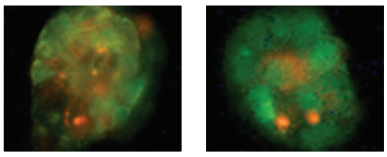
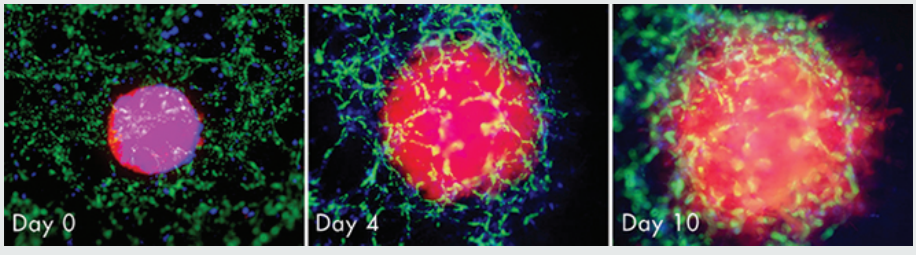


Nuclear morphology of MCF-10A, mammary epithelial acinar structures, as depicted using 1X SYBR Green reagent. 3-D structures were imaged using a Nikon Eclipse E400 microscope (100X magnification) using epifluorescence with a FITC filter and images were captured using a Q Imaging Micropublisher 3.3 camera.



Detection of luminal hollowing in MCF-10A, mammary epithelial acinar structures. Cells were labeled with 2 μM Calcein AM and 1 μM EtBr for 15 min. prior to imaging. Green cells indicate conversion of calcein AM to calcein by living cells, and red cells indicate compromised plasma membrane of dead cells.

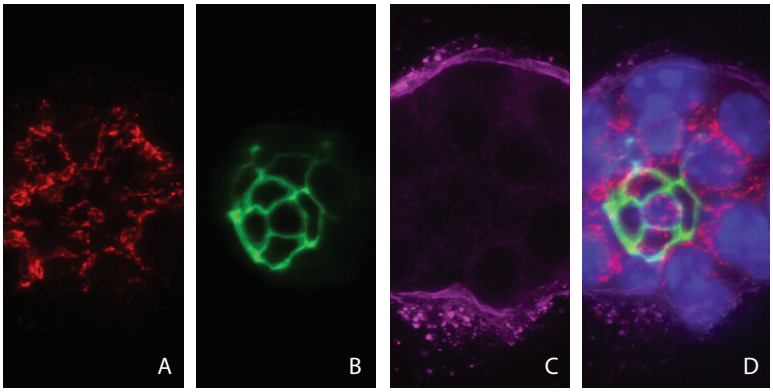


Formation of tri-culture structure on a mixture of Cultrex® ECM matrices, over 10 days. Red: breast cancer cell line (MDA-MB-231), Green: human umbilical vein endothelial cells (HUVECs); Blue: human adipose-derived mesenchymal stem cells (hMSCs).

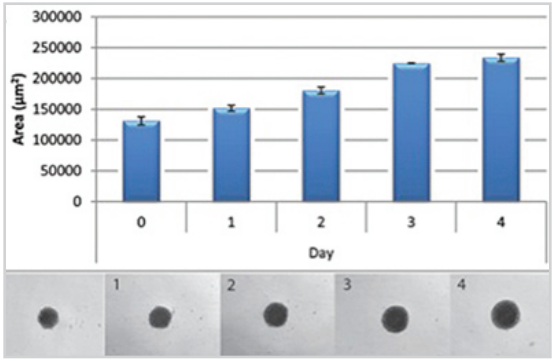
Supporting Data

3-D Culture Polarity

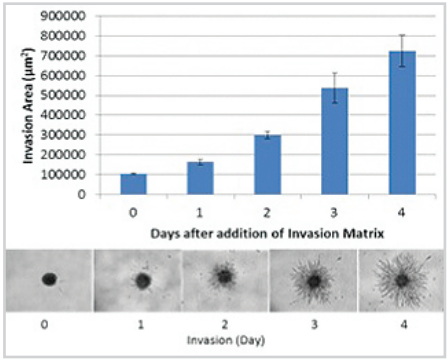
Non transgenic primary mammary cells grown in Cultrex® 3-D Culture Matrix develop into a polarized acinus. Confocal microscopy (5 µm projection) demonstrates epithelial polarity: DAPI stain, blue: GM130, red (Golgi protein, apical marker; panel A), ZO1, green (tight junctions, apical; panel B); Integrin a6, magenta (baso-lateral; panel C), overlay shown in panel D. Images courtesy of Martin Jechlinger.



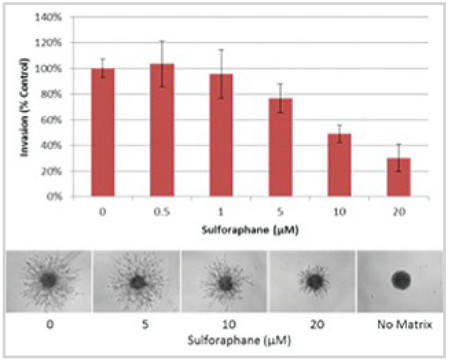
3-D Spheroid Assays



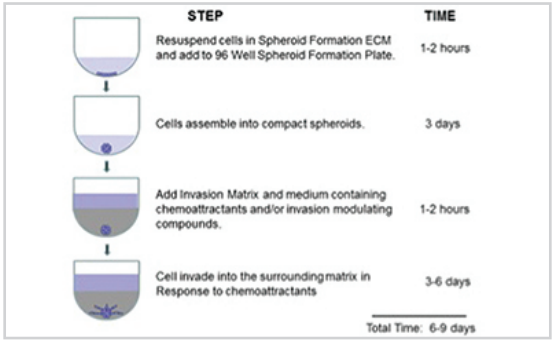
3-D culture proliferation of MDA-MB-231 breast cancer spheroids (using Cultrex® 3-D Spheroid Proliferation/Viability Assay) time lapse expansion of MDA-MB-231 spheroids over a 96 hour period.



Spheroid invasion by MDA-MB-231 breast cancer spheroids over a 96 hour period.

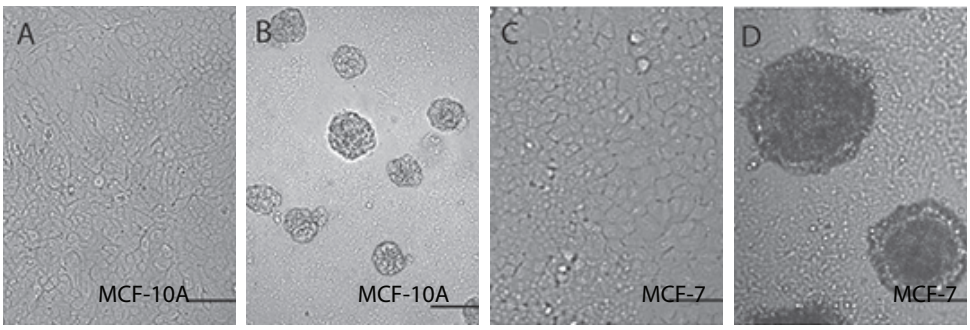


Inhibition of spheroid invasion by MDA-MB-231 breast cancer spheroids by Sulforaphane over a 96 hour period.



ImageJ analysis of spheroid invadabrachia.

3-D Morphology



Morphology of MCF-10A normal mammary epithelial cells in traditional 2-D (A) and 3-D BME (B) cell culture and MCF-7 mammary adenocarcinoma cells in traditional 2-D (C) and 3-D BME (D) cell culture, scale = 250 µm.

FAQs

What are 3-D cultures?

3-D cultures are *in vitro* cultures where immortalized cell lines, primary cell lines, stem cells, or explants are placed within hydrogel matrices that mimic *in vivo* cell environments.

What is the advantage of 3-D culture over traditional 2-D culture?

While 2-D culture has been used for studying many aspects of cell function and behavior, the tissue-culture treated plastic environment is unlike anything found within living organisms. As result, cells in 2-D culture exhibit altered morphology, function, proliferation and gene expression when compared to their emanating tissues. By placing these cells in a 3-D environment, they assume biological and biochemical characteristics similar to what is observed *in vivo*.

What are the variables associated with 3-D culture?

The major variables associated with 3-D culture are cell type, cell seeding density, composition of hydrogel, thickness of hydrogel, stiffness of hydrogel, composition of cell culture medium, and time of culture.

What are the different types of 3-D culture?

The two principal methods for conducting 3-D culture are the top assay and embedded assay. For the top assay, cells are seeded on a thick gel and a thin overlay is applied with the cell culture medium. For the embedded assay, cells are resuspended within a thick gel and the culture media is applied on top. The top assay is easier to setup, to control seeding densities, and to keep cells within one focal plane for analysis.

Which matrix should I use for 3-D culture?

Choice of matrix should correspond to the environment that you wish to recapitulate. A basement membrane extract (BME) will recapitulate the basal lamina, which underlie most cells of epithelial or endothelial origin. Collagen I is the major constituent of connective tissue, and it is commonly inhabited by stationary cells, such as fibrocytes and adipose cells, as well as migrating cells, such as mast cells, macrophages, monocytes, lymphocytes, plasma cells, and eosinophils.

How should cells be cultured prior to setting up the 3-D culture?

Cells need to be healthy and actively dividing in 2-D culture. Cells should be passaged two or three times after resuspension from cryopreservation, and they should never surpass 80% confluency during each passage. Cells should also be assessed using trypan blue, and they should exhibit less than 5% staining.

What type of analysis is typically applied to 3-D cultures?

Within the cultures, cells may be assessed for morphology, apical/basal polarity, protein localization, and relative proliferation. In addition, cells may be isolated from the 3-D culture and evaluated for levels of RNA and protein expression, as well as modifications to DNA.

Can I transfer organoids from one matrix to another?

We strongly recommend, as is the standard practice in cell culture, that you maintain the same matrix throughout your process of deriving and expanding your organoids. We've learned that intestinal and liver organoid culture is most successful using Cultrex® Basement Membrane Extract, Type 2 from start to finish rather than switching between matrices.

What is a recommended protocol for organoid culture?

- Air Liquid Interface (ALI) organoid cultures: an insert containing an acellular layer of ECM is used to suspend organoids above the culture medium level to create the air-liquid interface. ALI organoids include tissue stroma, and they usually utilize a collagen-1 ECM.

- Submerged organoid cultures: isolated epithelial stem cells are embedded in a BME Type 2 hydrogel that is positioned in the middle of a culture vessel. These stem cells routinely require medium containing Wnt, EGF, Noggin, and R-spondin-1 (WENR).

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