**References:**


1. Inch, W.R., J.A. McCredie, and R.M. Sutherland, *Growth of nodular carcinomas in rodents compared with multi-cell spheroids in tissue culture.* Growth, 1970. **34**(3): p. 271-82.
2. Folkman, J. and M. Hochberg, *SELF-REGULATION OF GROWTH IN THREE DIMENSIONS.* The Journal of Experimental Medicine, 1973. **138**(4): p. 745-753.
3. Sutherland, R.M., et al., *A multi-component radiation survival curve using an in vitro tumour model.* Int J Radiat Biol Relat Stud Phys Chem Med, 1970. **18**(5): p. 491-5.
4. Kawata, M., et al., *Neural Rosette Formation within in Vitro Spheroids of a Clonal Human Teratocarcinoma Cell Line, PA-1/NR: Role of Extracellular Matrix Components in the Morphogenesis.* Cancer Research, 1991. **51**(10): p. 2655-2669.
5. Kelm, J.M., et al., *Method for generation of homogeneous multicellular tumor spheroids applicable to a wide variety of cell types.* Biotechnology and Bioengineering, 2003. **83**(2): p. 173-180.
6. Ivascu, A. and M. Kubbies, *Rapid Generation of Single-Tumor Spheroids for High-Throughput Cell Function and Toxicity Analysis.* Journal of Biomolecular Screening, 2006. **11**(8): p. 922-932.
7. Vinci, M.M., *Advances in establishment and analysis of 3D tumour spheroid-based functional assays for target validation and drug evaluation.* BMC Biology, 2012. **10**(1): p. 29.
8. Kunz-Schughart, L.A., et al., *The Use of 3-D Cultures for High-Throughput Screening: The Multicellular Spheroid Model.* Journal of Biomolecular Screening, 2004. **9**(4): p. 273-285.
9. Sutherland, R.M., et al., *Oxygenation and Differentiation in Multicellular Spheroids of Human Colon Carcinoma.* Cancer Research, 1986. **46**(10): p. 5320-5329.
10. Hirschhaeuser, F., et al., *Multicellular tumor spheroids: An underestimated tool is catching up again.* Journal of Biotechnology, 2010. **148**(1): p. 3-15.


.

**10X Spheroid Formation ECM**
Cat#: 3500-096-01
Storage: -80 °C
1-800-873-8443

# CULTREX® Product Data

***For Research Use Only. Not For Use In Diagnostic Procedures***

## Cultrex® 10X Spheroid Formation ECM

**Catalog#:** 3500-096-01 **Size:** 600 µl

**Description:** Current *in vitro* tumor models lack either a physiological context and/or reproducible format for evaluating tumor cells *in vitro*. At present, the most popular method for compound screening and pathway analysis involves culturing cancer cells on rigid, tissue culture treated plastic surfaces where the cells adhere non-specifically and proliferate as a monolayer, and as a result, these cells lose both morphology and gene expression profiles associated with tumors *in vivo*. Alternatively, single cell suspensions may be embedded in extracellular matrix (ECM) hydrogels to construct 3-D cultures; however, the resulting structures are dispersed throughout the gel and exhibit significant variability in morphology and size, limiting the establishment of physiological gradients and adversely affecting the reproducibility of each assay.

To address issues of reproducibility and to build more physiological tumor systems, well-established methods for multicellular spheroid formation were incorporated into 3-D culture models. Researchers have been using spheroid cultures for cancer research for over 40 years[1-3]; however, there have been limitations regarding which cell lines could spontaneously form spheroids. For spontaneously spheroid assembly, it was shown that the cells produce an ECM that is deposited on the outer surface of the spheroid and that cell lines that could not spontaneously form compact spheroids were deficient or lacking in the formation of this ECM[4, 5]. It was later shown that the addition of ECM proteins to non-spheroid forming cells induced spontaneous spheroid formation, making the spheroid format compatible with most solid cancer cell models[6]. Trevigen has optimized this process, providing the necessary reagents to evaluate your cells using this method. Simply harvest cells, resuspend in spheroid formation ECM, and then culture in a 96 well spheroid formation plate. Spheroids generally form in 48 to 72 hours. Cell number and culture time determines spheroid size, and since each well produces one spheroid, researchers have complete control over spheroid dimensions with virtually no well to well variability. For most tumor models, we recommend spheroids between 400 – 500 µm in diameter. This is sufficient to establish physiological gradients for nutrients, oxygen, pH, and catabolites due to limitations in diffusion through the multicellular layers. Another effect of these gradients is the establishment of heterogeneous cell populations with necrotic cells in the core, quiescent cells in the deeper layers, and proliferating cells on the spheroid surface; all of these factors reminiscent of an avascular tumor[7-10]. Once formed, these multicellular tumor cell aggregates can be treated with pharmacological compounds to evaluate the effect on tumor spheroid growth; alternatively, specific genes or pathways may be manipulated to evaluate their effect on expansion of the *in vitro* tumor.



TREVIGEN®
8405 Helgerman Court, Gaithersburg, MD 20877 USA
Voice: 1-800-TREVIGEN (1-800-873-8443) • 301-216-2800
Fax: 301-560-4973 • e-mail: info@trevigen.com • www.trevigen.com

## Specifications:

Source: Murine Engelbreth-Holm-Swarm (EHS) tumor.
Storage Buffer: Dulbecco's Modified Eagle's medium (DMEM) containing 10 µg/ml gentamicin sulfate.
Storage/Stability: Product is stable for a minimum of 3 months from date of shipment. **Store at –80 °C; keep frozen. Dispense into working aliquots if needed; repeated freeze-thaws will destroy product integrity.**

## Material Qualification:

Functional Assay:

- Spheroid Formation Assay: supports spheroid formation for human breast cancer cells (MDA-MB-231) over a 72 hour period.

Sterility Testing:

- No bacterial or fungal growth detected after incubation at 37°C for 14 days following USP sterility testing guidelines.
- Endotoxin concentrations ≤ 8 EU/ml by LAL assay.

### Coating Procedures:

Refrigerator temperatures may vary; therefore thaw Cultrex® 10X Spheroid Formation ECM at 2-8°C overnight on ice in a refrigerator. 10X Spheroid Formation ECM may gel in 15-30 minutes above 15°C; keep the 10X Spheroid Formation ECM container on ice to prevent gelling. Dilute 10X Spheroid Formation ECM to 1X using cold (4 °C) cell culture medium; pipet up and down with a serological pipet to make a homogenous solution. Once in solution, resuspend cells in 1X Spheroid Formation ECM, and add solution to a 96 well spheroid formation plate. Centrifuge at 200 x g for 3 minutes, and incubate at 37 °C, 5% $CO_2$ to promote spheroid formation.

Spheroid Formation Overview (see 3510-096-K or 3511-096-K for detailed protocol):

1. Culture cells per manufacturer's recommendation; adherent cells should be cultured to no more than 80% confluence.
2. Thaw 10X Spheroid Formation ECM, catalog# 3500-096-01, on ice for two hours or overnight in a 4°C refrigerator.
3. Harvest cells and resuspend in 1X Spheroid Formation ECM.
4. Aliquot 50 µl of cell suspension per well of the 3D Culture Qualified 96 Well Spheroid Formation Plate, 3500-096-02, (not provided). Preserve unused wells for subsequent experiments by applying the strip seals that are included with each plate, if needed.
5. Centrifuge at 200 x *g* for 3 minutes at room temperature in a swinging bucket rotor.
6. Incubate at 37 °C in a tissue culture incubator for 72 hours to promote spheroid formation.

## Related Products:

| Catalog# | Description | Size |
|---|---|---|
| 3455-024-K | Cultrex® 24 Well BME Cell Invasion Assay | 24 inserts |
| 3480-024-K | CultreCoat® 24 Well BME-Coated Cell Invasion Assay | 24 inserts |
| 3456-024-K | Cultrex® 24 Well Laminin I Cell Invasion Assay | 24 inserts |
| 3457-024-K | Cultrex® 24 Well Collagen I Cell Invasion Assay | 24 inserts |
| 3458-024-K | Cultrex® 24 Well Collagen IV Cell Invasion Assay | 24 inserts |
| 3455-096-K | Cultrex® 96 well BME Cell Invasion Assay | 96 samples |
| 3465-024-K | Cultrex® 24 well Migration Cell Assay | 96 samples |
| 3465-096-K | Cultrex® 96 well Migration Cell Assay | 96 samples |
| 3456-096-K | Cultrex® Laminin I Cell Invasion Assay | 96 samples |
| 3457-096-K | Cultrex® Collagen I Cell Invasion Assay | 96 samples |
| 3458-096-K | Cultrex® Collagen IV Cell Invasion Assay | 96 samples |
| 3490-096-K | CultreCoat® BME 96 Well Cell Adhesion Assay | 96 samples |
| 3496-096-K | CultreCoat® 96 Well Adhesion Protein Array | 96 samples |
| 3450-048-SK | Cultrex® Directed In Vivo Angiogenesis Assay (DIVAATM) Starter | 48 samples |
| 3450-048-K | Cultrex® DIVAATM Kit | 48 samples |
| 3450-048-IK | Cultrex® DIVAATM Inhibition Kit | 48 samples |

## Accessories:

| Catalog# | Description | Size |
|---|---|---|
| 3400-010-02 | Cultrex® Mouse Laminin I, PathClear® | 1 ml |
| 3400-010-01 | Cultrex® Mouse Laminin I | 1 mg |
| 3440-100-01 | Cultrex® Rat Collagen I | 100 mg |
| 3410-010-01 | Cultrex® Mouse Collagen IV | 1 mg |
| 3420-001-01 | Cultrex® Human Fibronectin, PathClear® | 1 mg |
| 3416-001-01 | Cultrex® Bovine Fibronectin, NZHD* | 1 mg |
| 3421-001-01 | Cultrex® Human Vitronectin, PathClear® | 50 µg |
| 3417-001-01 | Cultrex® Bovine Vitronectin, NZHD | 50 µg |
| 3438-100-01 | Cultrex® Poly-L-Lysine | 100 ml |
| 3439-100-01 | Cultrex® Poly-D-Lysine | 100 ml |
| 3445-048-01 | Cultrex® 3-D Culture Matrix™ BME | 15 ml |
| 3446-005-01 | Cultrex® 3-D Culture Matrix™ Laminin I | 5 ml |
| 3447-020-01 | Cultrex® 3-D Culture Matrix™ Collagen I | 100 mg |
| 3430-005-01 | Cultrex® BME with Phenol Red | 5 ml |
| 3432-005-01 | Cultrex® BME without Phenol Red | 5 ml |
| 3431-005-01 | Cultrex® BME with Phenol Red; Reduced Growth Factor | 5 ml |
| 3433-005-01 | Cultrex® BME; no Phenol Red; Reduced Growth Factor | 5 ml |
| 3415-001-02 | Cultrex® Human BME, PathClear® | 1 ml |
| 3431-005-02 | Cultrex® BME with Phenol Red, Reduced Growth Factor PathClear® | 5 ml |
| 3432-005-02 | Cultrex® BME, PathClear® | 5 ml |
| 3433-005-02 | Cultrex® BME; no Phenol Red; Reduced Growth Factor PathClear® | 5 ml |
| 3437-100-K | Cultrex® Cell Staining Kit | 100 ml |
| 3450-048-05 | CellSperse™ | 15 ml |

***New Zealand Herd Derived**