

References:

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**10X Spheroid Formation
ECM**

Cat#: 3500-096-01

Storage: -80 °C

1-800-873-8443

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.

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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 \leq 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% CO₂ to promote spheroid formation.

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

- Culture cells per manufacturer's recommendation; adherent cells should be cultured to no more than 80% confluence.
- Thaw 10X Spheroid Formation ECM, catalog# 3500-096-01, on ice for two hours or overnight in a 4°C refrigerator.
- Harvest cells and resuspend in 1X Spheroid Formation ECM.
- 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.
- Centrifuge at 200 x g for 3 minutes at room temperature in a swinging bucket rotor.
- 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	CellSpense™	15 ml

*New Zealand Herd Derived