

References:

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CULTREX® Product Data*For Research Use Only. Not For Use In Diagnostic Procedures***Cultrex® Invasion Matrix and 3D Culture Qualified 96 Well Spheroid Formation Plate**

Catalog#:	3500-096-IP	Size:	6 ml
Contents:	3500-096-03		Each
	3500-096-02		

Description: The 96 Well 3D Spheroid BME Cell Invasion Assay offers a standardized, three-dimensional, high content format for quantitating the degree to which invasive cells penetrate a barrier, consisting of basement membrane components, *in vitro* in response to chemoattractants and/or inhibiting compounds, which is fundamental for angiogenesis¹, embryonic development², immune response³, and tumor cell metastasis⁴. Basement membranes are continuous sheets of specialized extracellular matrix that form an interface between endothelial, epithelial, muscle, or neuronal cells and their adjacent stroma. They not only support cells and cell layers, but they also play an essential role in tissue organization that influences cell adhesion, migration, proliferation, and differentiation. Basement membranes are degraded and regenerated during development and wound repair, and they are major barriers to invasion by metastatic tumor cells.

The need for more complete and physiologically predictive cancer invasion models has driven the development of the 96 Well 3D Spheroid BME Cell Invasion Assay. There is growing evidence that tumor cell aggregates or spheroids are more representative of tumors *in vivo*, and they exhibit several physiological traits including similar morphology, the formation of cell-cell bonds, decreased proliferation rates, increased cell survival, tumor dormancy, and a hypoxic core⁵⁻⁸. Applying this model to a 3D culture invasion assay provides a more physiological approach for assessing tumor invasion and offers a visual component that can be quantitated through image analysis.

Upon completion of spheroid formation, the spheroid is embedded in Invasion Matrix composed of extracellular matrix proteins. This matrix forms a hydrogel network on which invasive cells can travel. At this point, invasion modulating agents can be applied to the system to evaluate the impact on cell response. Cell invasion is visualized microscopically and can be quantitated through image analysis software.

Specifications:

<u>Source:</u>	Murine Engelbreth-Holm-Swarm (EHS) tumor and bovine extensor tendon.
<u>Storage Buffer:</u>	Dulbecco's Modified Eagle's medium (DMEM) containing 10 µg/ml gentamicin sulfate.
<u>Storage/Stability:</u>	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.



**Cultrex® Invasion Matrix and
3D Culture Qualified 96 Well
Spheroid Formation Plate**

Cat#: 3500-096-SP
Storage: -80 °C
1-800-873-8443

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Material Qualification:

Functional Assay:

- Spheroid Invasion Assay: supports invasion of cancer cells (MDA-MB-231 human breast cancer cells or HT-1080 human fibrosarcoma) out of a spheroid over a 96 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® Invasion Matrix at 2-8°C overnight on ice in a refrigerator. Invasion Matrix gels in 15-30 minutes above 15°C; keeping the Invasion Matrix container and coated labware on ice will prevent gelling and extend working times. Gently inverted Invasion Matrix to make a homogenous solution. Bubbles may be prevented or eliminated from the Invasion Matrix by maintaining labware on ice during coating and centrifuging 300 x g for 10 minutes at 4°C.

Spheroid Invasion Overview (see 3500-096-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, (not provided) 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. 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.
7. Thaw Invasion Matrix on ice for four hours or overnight in a 4°C refrigerator.
8. Place 3D Culture Qualified 96 Well Spheroid Formation Plate on ice in refrigerator for 15 minutes to cool wells.
9. Working on ice, add 50 µl of Invasion Matrix per well of the 3D Culture Qualified 96 Well Spheroid Formation Plate. Centrifuge plates at 300 x g at 4°C for 5 minutes in a swinging bucket rotor to eliminate bubbles and position spheroids within the Invasion Matrix towards the middle of the well. **Caution: centrifugation at high speeds and/or extended periods of time can adversely impact subsequent spheroid invasion.**
10. Transfer plate to a tissue culture incubator set at 37 °C for one hour to promote gel formation.
11. After one hour, add 100 µl of cell culture medium containing chemoattractant and invasion modulating compounds, if applicable.
12. Incubate at 37 °C in a tissue culture incubator for 3 to 6 days, and photograph

the spheroid in each well every 24 hours using the 4X objective.

13. Analyze images to evaluate 3D culture cell invasion.

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