CULTREX® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

CultreCoat® 24 Well Low BME Cell Invasion Inserts

Catalog #: 3481-024-01 **Size:** 24 Inserts in Two 24 Well Plates

Description: Cell invasion is fundamental to angiogenesis[1], embryonic development[2], immune responses[3], and tumor cell metastasis[4]. Trevigen's **CultreCoat® 24 Well Cell Invasion Assays** were created in an effort to accelerate *in vitro* screening for modulators of these processes, and to further enable the investigation of pathways that influence cell invasion through extracellular matrices. These assays offer three distinct, standardized, high throughput formats for quantitating the degree to which invasive cells penetrate a barrier consisting of basement membrane components, in response to chemoattractants and/or inhibiting compounds. The **CultreCoat® 24 Well Low BME Cell Invasion Inserts** have a polycarbonate membrane with 8 µm pores; they are coated with a thin basement membrane that is intended for use with minimally invasive cell lines. The inserts are provided by themselves for researchers that have designed their own assay system and detection method; a complete kit containing all assay and detection reagents is also available (catalog number 3481-024-K).

Storage: Store at -20 °C in a manual defrost freezer.

Recommended Procedure for CultreCoat® 24 Well Low BME Cell Invasion Inserts:

Note: These are general recommendations; cell numbers, incubation periods, medium compositions and volumes may be adjusted as needed or desired.

Note: All steps prior to "Detection" should be conducted in sterile conditions.

Prior to Starting the Assay

1. Culture cells per manufacturer's recommendation. Adherent cells should be passaged at least one time and cultured to 80% confluence. Cells should be dividing in log phase, and viability should be greater than 90%. Plan accordingly for sufficient numbers of cells per well.
2. If the cells require serum starvation, this needs to be performed prior to setting up the assay. Cells are routinely starved for 16-24 hours prior to assay in a serum-free medium (0.5% FBS may be used if needed to maintain viability).

Cell Invasion

3. Transfer the 24 well cell invasion inserts to room temperature, and acclimate for one hour.
4. Rehydrate the inserts by adding 80 µl of serum-free medium (~37 °C) to each insert, and incubating at 37 °C in a CO_2 incubator for 1 hour.
5. After serum starvation (if applicable), harvest and count cells.
6. Dilute to working concentration (1 x 10^6 cells/ml is a recommended starting point) in a serum-free medium (0.5% FBS may be used if needed). Inhibitors may also be added to cells at this time.
7. After rehydration, add 40 µl of cells per well to each insert (40,000 cells recommended).
8. Add 360 µl of medium per well to bottom well (with or without chemoattractants and/or inhibitors).
9. Assemble chambers and incubate at 37 °C in a CO_2 incubator for 4-48 hours (24 hours recommended).

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Detection

10. Upon completion of invasion, use a cotton-tipped swab to remove non-invading cells from the top of the membrane. Gently swab the entire membrane and be careful not to puncture the polycarbonate membrane.
11. Label the invaded cells. Some labels require cells to be fixed prior to labeling while others may necessitate viable cells. Optimal labeling concentrations may also differ. For fluorescent labeling, we recommend Calcein AM (4892-010-K) at 2 µM, or for colorimetric labeling, we recommend Cell Staining Kit (3437-100-K). Consult manufacturer for recommended conditions.
12. Photograph cells that have attached to the bottom of the membrane and count cells within each field to determine relative invasiveness.

References

1. Tamilarasan KP, Kolluru GK, Rajaram M, Indhumathy M, Saranya R, Chatterjee S. 2006. Thalidomide attenuates nitric oxide mediated angio-genesis by blocking migration of endothelial cells. *BMC Cell Biol.* **7**:17.
2. Borghesani PR, Peyrin JM, Klein R, Rubin J, Carter AR, Schwartz PM, Luster A, Corfas G, Segal RA. 2002. BDNF stimulates migration of cerebellar granule cells. *Development* **129**:1435-1442.
3. Mohan K, Ding Z, Hanly J, Issekutz TB. 2002 IFN-gamma-inducible T cell alpha chemoattractant is a potent stimulator of normal human blood T lymphocyte transendothelial migration: differential regulation by IFN-gamma and TNF-alpha. *J Immunol.* **168**:6420-6428.
4. Li G, Chen YF, Greene GL, Oparil S, Thompson JA. 1999 Estrogen inhibits vascular smooth muscle cell-dependent adventitial fibroblast migration *in vitro*. *Circulation* **100**:1639-1645.

Related Products:

Catalog#	Description	Size
3455-024-K	Cultrex® 24 Well BME Cell Invasion Assay	24 samples
3456-024-K	Cultrex® 24 Well Laminin I Cell Invasion Assay	24 samples
3457-024-K	Cultrex® 24 Well Collagen I Cell Invasion Assay	24 samples
3458-024-K	Cultrex® 24 Well Collagen IV Cell Invasion Assay	24 samples
3465-024-K	Cultrex® 24 Well Cell Migration Assay	24 samples
3480-024-K	CultreCoat® 24 Well BME Coated Cell Invasion Assay	24 samples
3484-024-K	CultreCoat® 24 Well Cell Invasion Optimization Assay	24 samples
3455-096-K	Cultrex® 96 Well BME Cell Invasion Assay	96 samples
3456-096-K	Cultrex® 96 Well Laminin I Cell Invasion Assay	96 samples
3457-096-K	Cultrex® 96 Well Collagen I Cell Invasion Assay	96 samples
3458-096-K	Cultrex® 96 Well Collagen IV Cell Invasion Assay	96 samples
3465-096-K	Cultrex® 96 Well Cell Migration Assay	96 samples
3484-096-K	CultreCoat® 96 Well Cell Invasion Optimization 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

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1-800-873-8443