CULTREX® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

amsbio

In Vitro Angiogenesis Assay Endothelial Cell Invasion

Reagent kit for investigating migration and/or invasion of endothelial cells.

96 Samples

Catalog #: 3471-096-K

Cultrex® *In Vitro* Angiogenesis Assay Endothelial Cell Invasion

Catalog# 3471-096-K

96 Samples

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I. Quick Reference Procedure for Cultrex® *In Vitro* Angiogenesis Assay: Endothelial Cell Invasion (Cat# 3471-096-K): Read through the complete Instructions for Use prior to using this kit.

Prior to Day 1

1. Culture cells per manufacturer's recommendation; endothelial cells should be cultured to no more than 80% confluence. Each well requires 20,000 cells on average, so plan accordingly.
2. Coat membranes of top invasion chamber with 50 µl of 0.1X to 1X BME Coating Solution or 1X Coating buffer (as a migration control) and incubate for 4 hours or overnight at 37° C in a CO_2 incubator.

Day 1

4. Harvest and count endothelial cells.
5. Centrifuge cells at 200 x g for 3 min, remove supernatant and resuspend at 4 x 10^5 cells/ml in a serum-free medium.
6. Aspirate top chamber of cell invasion device. DO NOT ALLOW TOP OR BOTTOM CHAMBERS TO DRY.
7. Add 50 µl cell suspension (20,000 cells) per well to top chamber (with or without inhibitors/stimulants).
8. Using access port, add 150 µl of medium per well to bottom chamber (with or without chemoattractants and inhibitors).
9. Incubate chamber at 37° C in CO_2 incubator for 24 hours.
10. Assay remaining cells for standard curve (section VII. B.) (optional); each cell type will require a separate standard curve (optional).

Day 2

11. Carefully aspirate top chamber (do not puncture membrane), and wash each well with 100 µl of 1X Wash Buffer.
12. Aspirate bottom chamber, and wash each well twice with 200 µl 1X Wash Buffer. Transfer tops to assay chamber plate (black).
13. Add 10 µl of 2 mM Calcein-AM working solution to 10 ml of Cell Dissociation Solution.
14. Add 150 µl of Cell Dissociation Solution/Calcein-AM to bottom chamber, assemble cell invasion device, and incubate at 37° C in CO_2 incubator for one hour.
15. Remove top chamber, and read the black bottom plate at 485 nm excitation, 520 nm emission.
16. Using standard curve(s), convert RFU to Cell Number; determine percent invasion.
17. Cell invasion can be represented as a percent of migration (100% - value of migration in uncoated wells in the presence of chemoattractants).

II. Background

Angiogenesis is the process of forming new capillaries from post-capillary venules and, *in vivo*, it is driven by tissue hypoxia and many angiogenic factors. Angiogenesis contributes to embryonic development (vasculogenesis), homeostasis, wound healing, and tumor development, growth and survival (1-4). The initiation of an angiogenic response involves recruited vascular endothelial cells, which degrade the extracellular matrix as they invade hypoxic tissue (2, 3). This breakdown occurs through the regulated release and activation of specific matrix metalloproteinases (MMP), such as MMP-2, and MMP-9 (4-6). MMP-9 promotes vascular endothelial cell invasion upon stimulation by protein kinase C activation (7) or thrombospondin-1 (6) and promotes endothelial cell morphogenesis (7). Furthermore, tumor angiogenesis is inhibited in MMP-9 null mouse hosts, even though the tumor cells originate from a wild type, MMP-9 producing strain (8). Once the extracellular matrix barrier has been traversed, vascular endothelial cells proliferate and migrate toward the source of chemotactic stimuli, forming an endothelial cord. These cords connect to form lumens that can eventually develop into vessels.

Trevigen's Cultrex® *In Vitro* Angiogenesis Assay: Endothelial Cell Invasion was created in an effort to accelerate the screening process for compounds that influence vascular endothelial cellular digestion through and migration across a basement extracellular membrane (BME). This kit offers a flexible, standardized, high-throughput format for quantitating the degree to which endothelial cells penetrate an *in vitro* BME barrier in response to chemoattractants and/or inhibiting compounds. This assay employs a simplified Boyden chamber-like design with an 8 micron polyethylene terephthalate (PET) membrane. Ports within the migration/invasion chamber (top) allow access to the assay chamber (bottom) without dismantling the device. This design is easier to use, prevents contamination, and is adaptable for robotic high-throughput systems. The assay chamber may be directly analyzed in a 96 well plate reader, eliminating transfer steps that introduce additional variability to the assay. The permissiveness of the BME matrix may also be optimized to fit each experiment by adjusting the coating concentration.

Sulforaphane [1-isothiocyanato-(4R)-methylsulfinyl)-butane], found in broccoli and other cruciferous vegetables, is a naturally occurring cancer chemopreventive agent, and is provided as a control for inhibition of *in vitro* endothelial cell migration/invasion on Cultrex® Basement Membrane Extract.

Detection of cell invasion is quantified using Calcein-AM. Calcein-AM is internalized by endothelial cells, and intracellular esterases cleave the acetomethylester (AM) moiety to generate free Calcein. Free Calcein fluoresces brightly, and this fluorescence may be used to quantitate the number of cells that have migrated or invaded using a standard curve. Sufficient reagents are included to assess cell migration/invasion in 96 wells, as well as to calculate multiple standard curves.

III. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of Trevigen's ***In Vitro* Angiogenesis Assay: Endothelial Cell Invasion kit** may not yet have been fully investigated therefore Trevigen recommends the use of gloves, lab-coats, and eye protection while using these chemical reagents. Trevigen assumes no liability for damage resulting from handling or contact with these products.

3. This kit contains reagents that may be harmful if swallowed, or come in contact with skin or eyes. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Material safety data sheets are available on request.

IV. Materials Supplied

Component	Quantity	Storage	Catalog#
Cell Invasion Chamber	each	Room Temp	3455-096-01
5X BME Solution	1 ml	≤-20° C*	3455-096-02
10X Coating Buffer	1 ml	4° C	3455-096-03
25X Cell Wash Buffer	2 x 1.5 ml	4° C	3455-096-04
10X Cell Dissociation Solution	2 x 1.5 ml	Room Temp	3455-096-05
Calcein AM	50 µg	≤-20° C	4892-010-01
Sulforaphane, 10 mM	15 µl	≤-20° C	3470-096-02

*Store in a manual defrost freezer; for long term storage, store at -80° C.

V. Materials/Equipment Required But Not Supplied

Equipment

1. 1 - 20 µl, 20 - 200 µl, and 200 - 1000 µl pipettors
2. 37° C CO_2 incubator
3. Low speed centrifuge and tubes for cell harvesting
4. Hemocytometer or other means to count cells
5. -20° C and 4° C storage
6. Ice bucket
7. Standard light microscope (or inverted)
8. Timer
9. Fluorescent 96-well plate reader, top reader (485 nm excitation, 520 nm emission)
10. Black 96 Well Plate (For standard curve)
11. Computer and graphing software, such as Microsoft® Excel®.

Reagents

1. Cell Harvesting Buffer; EDTA, trypsin, or other cell detachment buffer.
2. Tissue Culture Growth Media, as recommended by cell supplier.
3. Serum-Free Media, Tissue Culture Growth Media without serum.
4. Chemoattractants or pharmacological agents for addition to culture medium.
5. Sterile PBS or HBSS.
6. Distilled, deionized water
7. Trypan blue or equivalent viability stain

Disposables

1. Cell culture flask, 25 cm^2 or 75 cm^2
2. 50 ml tubes
3. 1 - 200 µl and 200 - 1000 µl pipette tips
4. 2, 5 and 10 ml serological pipettes
5. Gloves
6. 10 ml syringe
7. 0.2 µm filter

VI. Reagent Preparation

(Thaw reagents completely before diluting!)

1. 1X Coating Buffer
Add 0.5 ml of 10X Coating Buffer to 4.5 ml of sterile, deionized water to make 5 ml 1X Coating Buffer; filtration at 0.2 µm recommended. Store at 4° C.

2. 1X BME Solution
For more invasive cells add 1 ml of 5X BME to 4 ml of 1X Coating Buffer on ice immediately before coating (800 µg/cm^2). Less invasive cell types may require a more permissive barrier, so the BME may be diluted as far as 0.1X (80 µg/cm^2). Avoid freeze-thaws.

3. 1X Cell Wash Buffer
Add 3 ml of 25X Cell Wash Buffer to 72 ml of sterile, deionized water to make 75 ml of 1X solution.

4. 1X Cell Dissociation Solution
Add 3 ml of 10X Cell Dissociation Solution to 27 ml of sterile, deionized water to make 30 ml of 1X solution.

5. 2 mM Calcein AM Working Solution
Centrifuge microtube momentarily to pellet powder before opening tube, and add 25 µl of sterile DMSO to make 2 mM working solution. Pipet up and down to mix, and store solution at –20 ^oC.

6. 100 µM Sulforaphane Working Solution:
Add 2 µl of 10 mM Sulforaphane to 198 µl of Endothelial Basal Medium (EBM) or sterile PBS.

VII. Assay Protocol

These procedures should be performed in a biological hood utilizing aseptic technique to prevent contamination.

A. Cell Harvesting

Subject cells may be prepared for investigation as desired. The following procedure is suggested and may be optimized to suit the endothelial cell type(s) of interest.

1. Cells should be passaged 1 or 2 times prior to use in the assay. Endothelial cells show best results when plated 24 hours prior to assay and do not exceed greater than 80% confluence. Each chamber requires 20,000 cells, and a 25 cm^2 or 75 cm^2 flasks will yield approximately 2 x 10^6 or 8 x 10^6 cells, respectively. Plan to have enough cells for a standard curve, if used, migration controls and cell invasion assay.
2. Prior to harvest, visually inspect cells, and record cell health, relative number, and morphology.
3. Wash cells two times with sterile PBS or HBSS. Use 5 ml per wash for a 25 cm^2 flask and 10 ml per wash for a 75 cm^2 flask.

4. Harvest cells. For 25 cm^2 or 75 cm^2 flask, add 1 ml or 3 ml, respectively, of Trypsin-EDTA or another Cell Harvesting Buffer (see *Materials/Equipment Required But Not Supplied*), and incubate at 37° C for 1 to 5 minutes (until cells have dissociated from bottom of flask).
5. Transfer cells to a 15 ml conical tube, and add 1 ml or 3 ml, respectively, of FBS-containing medium to inhibit trypsin.
6. Centrifuge cells at 200 x *g* for 3 minutes to pellet, remove quenching medium, and resuspend cells in 1 ml of Serum-Free Medium. Cells may need to be gently pipetted up and down using a serological pipette to break up clumps.
7. Count cells, and dilute to 4 x 10^5 cells per ml in Serum-Free Medium.

B. Conversion of Relative Fluorescence Units (RFU) into Cell Number

Many investigators express their results relative to total number of cells loaded per well. In order to convert relative fluorescence units (RFU) into number of cells, standard curves are recommended. It is not necessary to use inserts to generate a standard curve. If used, a separate standard curve may be run for each cell type and assay condition. Control cells, and experimental replicates should be performed at least in triplicate.

1. Determine the saturation range for your cells, beyond which additional migration or invasion would be difficult to detect, and the maximum range of the standard curve (e.g. 50,000, 20,000, 10,000, 5,000 and 2,000 cells).
2. Determine the total number of cells needed per standard curve, and the required volume of medium (Table 1, below).
   = cells/well x wells/condition = cells/condition
   Sum (cell/condition) = total number of cells
3. Calculate the volume of harvested cells needed (see Table 1, below).
   = total number of cells needed/1 x 10^6 cells/ml
4. Transfer volume of harvested cells needed to a 15 ml conical tube, and centrifuge at 200 *x g* for 3 minutes to pellet cells.
5. Remove supernatant, and resuspend cells in 1X Cell Dissociation Solution at 1.0 x 10^6 cells/ml.
6. Dilute cells for highest concentration for a final volume of 50 µl (e.g. 50,000 cells/ 50 µl = 1.0 x 10^6 cells/ml) in 1X Cell Dissociation Solution (section VI, item 4), add 50 µl per well, and serially dilute remaining stock with 1X Cell Dissociation Solution to deposit the number of cells needed in each well (in 50 µl of 1X Cell Dissociation Solution). Repeat dilutions until all conditions have been satisfied.
7. Add 10 µl of 2 mM Calcein AM Working Solution (section VI, item 5) to 5 ml of 1X Cell Dissociation Solution (section VI, item 4), cap tube, and invert to mix.
8. Add 50 µl of Calcein AM/Cell Dissociation Solution (section VII, step B6) to each well, and incubate for one hour; omit cells from at least three wells to calculate background.
9. Read plate at 485 nm excitation, 520 nm emission (see Table 2 for sample data) to obtain relative fluorescence units (RFU).
10. Average values for each condition; then subtract background from each value (see Table 2).
11. Plot standard curve of RFU vs. number of cells (see Figure 1).
12. Insert a trend line (best fit) and use the line equation for each cell line in calculating number of cells that migrated/invaded (see Figure 1).

Table 1. Sample Calculations for Standard Curve:

Cells Needed:

Cells/Well	X Conditions	= Cells Needed
50,000	3	150,000
20,000	3	60,000
10,000	3	30,000
5,000	3	15,000
2,000	3	6,000
Total Cells Needed		**161,000**

Volume of Harvested Cells Needed (ml):

$$\frac{\text{Total Cells Needed}}{\text{Concentration Harvested Cells}} = \frac{161{,}000}{1.00\text{E+06}} = 0.161$$

Table 2. Sample Data for Standard Curve (actual results may vary):

Average Cells/Well	Wells 1	2	3
50,000	45349	44528	42904
20,000	14875	15316	15437
10,000	9616	8497	9521
5,000	2515	2649	2509
2,000	992	988	988
0	320	332	306

Subtract Background and plot the data:

Cells/Well	Average	Background	Corrected
50,000	44260	-319	43941
20,000	15209	-319	14890
10,000	9211	-319	8890
5,000	2558	-319	2238
2,000	989	-319	670
0	319	(Background)	

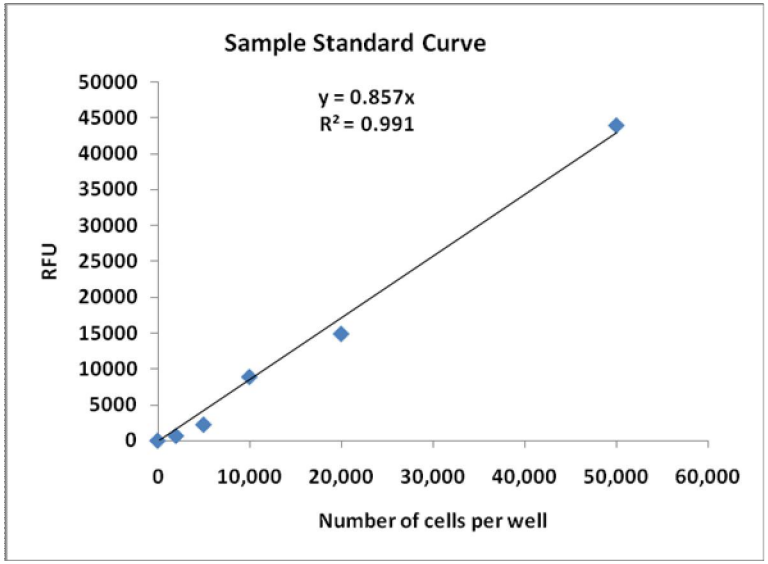

Figure 1. Standard Curve for Endothelial Cell Invasion Assay. SVEC4-10 cells were harvested (section VII. A), diluted, incubated for one hour with Calcein-AM, and assayed for fluorescence (section VII. B). The trend line and line equation are included on the graph.

C. Cell Invasion Assay

Note: Two plate bottoms are provided for your convenience: optimization of assay conditions (clear), and background signal minimization (black).

1. Working on ice, prepare 5 ml of 0.1X to 1X BME Solution in a sterile 15 ml conical tube (section VI, item 2), and label "BME Coat". Cap tube, and gently invert to mix.
2. Aliquot 50 µl of BME Coat (Section VII, C1) per well to top chamber of cell invasion device to measure cell invasion.
3. Aliquot 50 µl of 1X Coating Buffer per well to top chamber of cell invasion device to measure cell migration.
4. Gently tap side of Cell Invasion Chamber a few times, and visually inspect wells for dispersion of coating.
5. Incubate coated Cell Invasion Chamber for 4 hours or overnight at 37° C in CO_2 incubator.
6. **Optional:** Assay cells for standard curve; each cell type will require a separate standard curve (section VII. B).
7. Carefully aspirate off coating solution from top chamber of cell invasion device. DO NOT ALLOW TOP OR BOTTOM CHAMBER TO DRY.
8. Harvest cells, and dilute to working concentration (4 x 10^5 cells/ml recommended) in a serum-free growth factor-free medium (e.g. Endothelial Basal Medium).
9. Add 50 µl of cells per well (20,000 cells/well) to top chamber (with or without inhibitors/stimulants), and to compensate for background, omit cells from at least three wells.

10. Add 150 µl of medium per well to bottom chamber with (e.g. Endothelial Growth Medium-2) or without (e.g. Endothelial Basal Medium) chemo-attractants and in the presence or absence of inhibitor (e.g. Sulforaphane).
11. Incubate at 37° C in CO_2 incubator; incubation times may be varied (24 hours - 48 hours).
12. After incubation, aspirate top chamber without puncturing the membrane, and wash each well with 100 µl of 1X Wash Buffer (section VI, item 3). Aspirate out the wash buffer.
13. Aspirate bottom chamber, and wash each well with 200 µl 1X Wash Buffer (section VI, item 3). The device may be disassembled and the bottom chamber inverted to empty wells. Re-assemble device (if disassembled) using the assay chamber plate for minimal background signal.
14. Add 10 µl of Calcein-AM solution (section V, item 5) to 10 ml of 1X Cell Dissociation Solution (section VI, item 4).
15. Add 150 µl of Cell Dissociation Solution/Calcein AM (VII.C.14) to each bottom chamber well, and incubate at 37 ^oC in CO_2 incubator for 30 minutes.
16. Gently tap device 10 times on the side, and incubate at 37 ^oC in CO_2 incubator for an additional 30 minutes (one hour total). This helps to ensure optimal dissociation.
17. Disassemble cell migration device, and read assay chamber (bottom) at 485 nm excitation, 520 nm emission using same parameters (time and gain) as standard curve(s), or controls (see Table 3 for sample data).
18. Compare data to standard curve to determine the number of cells that have migrated (coating buffer) or invaded (through the BME).
19. Cell invasion can also be represented as a percent of migration (100% - value of migration in uncoated wells in the presence of chemoattractants) (see VIII. Example Results).

VIII. Example Results (actual results may vary)

1. After plotting standard curve (section VII. B), insert trendline, best fit, with intercept equals zero, equation, and R-square value (coefficient of determination).
2. For assay samples, first average all wells for each condition (Table 4).
3. Next, subtract background from averages (Table 5).
4. Use the trendline equation to determine the number of cells present in each well; for the equation, y = mx + b, replace Y value with RFU, and solve for x (Table 6). See an example of a trendline and equation in Figure 1.
5. The number of cells may be compared for each condition to evaluate relative invasion, or the number of invaded cells may be divided by the number of starting cells to determine percent invasion (Table 7 and Figure 2).
6. Cell invasion can also be represented as a percent of migration (100% - value of migration in uncoated wells in the presence of chemoattractants) (Table 8 and Figure 3)

Table 3. Sample data for Cell Invasion Assay (Actual Results May Vary):

Cell Line	Std Curve Slope (m value)
SVEC4-10	0.857
HUVEC	0.792
HBMVEC	0.915

Table 3 (continued):

		EBM			EGM-2			EGM-2 + 5 µM Sulforaphane		
SVEC4-10	Coating Buffer	414	428	497	5299	5604	5201	2006	2136	2253
	1X BME Coat	278	306	301	2268	2530	2152	1462	1393	1117
HUVEC	Coating Buffer	712	715	689	4261	4271	4109	1521	1832	1860
	1 X BME Coat	825	880	778	1991	1859	2043	728	717	809
HBMVEC	Coating Buffer	537	487	444	7867	8071	7963	1761	1922	2199
	1 X BME Coat	646	680	504	4694	5439	5353	1165	1152	1459
	Empty wells	145	140	140	133	158	159	154	149	158

Table 4. Average Data for Each Condition on 1X BME coated wells:

	EBM		EGM-2		EGM-2 + 5 µM Sulforaphane	
	average	standard deviation	average	standard deviation	average	standard deviation
SVEC 4-10	**295**	15	**2317**	194	**1324**	183
HUVEC	**828**	51	**1964**	95	**715**	50
HBMVEC	**610**	93	**5162**	408	**1259**	174

Table 5. Subtract Background from Each Condition:
Background (average RFU on wells of empty row) = 149

	EBM		EGM-2		EGM-2 + 5 µM Sulforaphane	
	average	standard deviation	average	standard deviation	average	standard deviation
SVEC 4-10	**146**	15	**2168**	194	**1175**	183
HUVEC	**679**	51	**1815**	95	**602**	50
HBMVEC	**461**	93	**5013**	408	**1110**	174

Table 6. Divide each value by line equation to determine cell number:

	EBM		EGM-2		EGM-2 + 5 µM Sulforaphane	
	average	standard deviation	average	standard deviation	average	standard deviation
SVEC 4-10	**170**	17	**2529**	226	**1371**	213
HUVEC	**857**	64	**2292**	120	**761**	63
HBMVEC	**504**	102	**5479**	445	**1213**	190

Table 7. Divide Cell Number for Each Condition by 20,000 to determine percent invasion:

	EBM		EGM-2		EGM-2 + 5 µM Sulforaphane	
	average	standard deviation	average	standard deviation	average	standard deviation
SVEC 4-10	**0.85**	0.09	**12.65**	1.13	**6.86**	1.07
HUVEC	**4.28**	0.32	**11.46**	0.06	**3.80**	0.32
HBMVEC	**2.52**	0.51	**27.39**	2.23	**6.06**	0.95

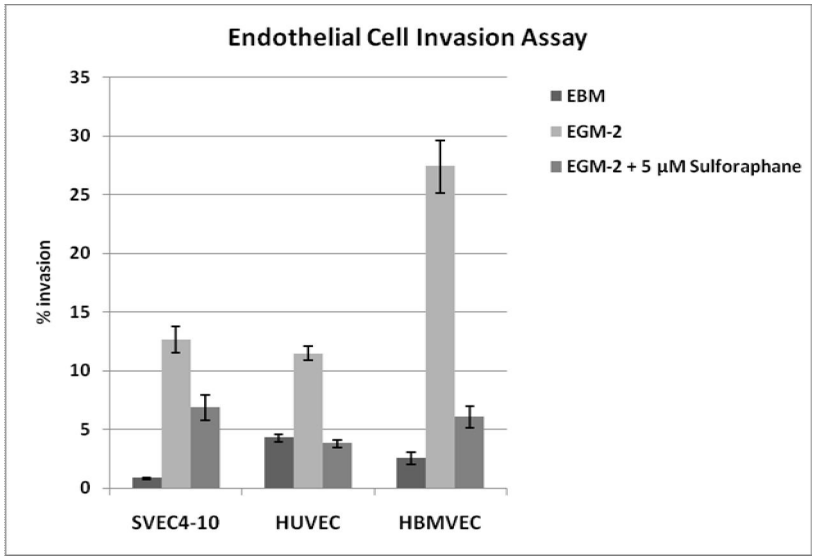

Figure 2. Quantification, by standard curve, of the ability of endothelial cell lines (SVEC4-10, HUVEC and HBMVEC) to cross a barrier consisting of an 8 micron polyester filter coated with 1 X BME (basement membrane extract) over a 24 hours period in response to FBS, VEGF, bFGF and other growth factors containing in the EGM-2, in the presence or absence of 5 µM Sulforaphane using standard curve. Y axis represent percent invasion.

Table 8. Determine percent invasion relative to migration (migration–100%):

		EBM		EGM-2		EGM-2 + 5 µM Sulforaphane	
		average	standard deviation	average	standard deviation	average	standard deviation
SVEC4-10	Coating Buffer	**5.70**	0.85	**100.00**	4.03	**37.99**	2.37
	1X BME Coat	**2.80**	0.29	**41.53**	3.71	**22.51**	3.50
HUVEC	Coating Buffer	**13.69**	0.35	**100.00**	2.23	**39.08**	4.63
	1X BME Coat	**16.70**	1.26	**44.66**	2.33	**14.82**	1.24
HBMVEC	Coating Buffer	**4.35**	0.60	**100.00**	1.31	**22.64**	2.58
	1X BME Coat	**5.90**	1.19	**64.12**	5.21	**14.19**	2.28

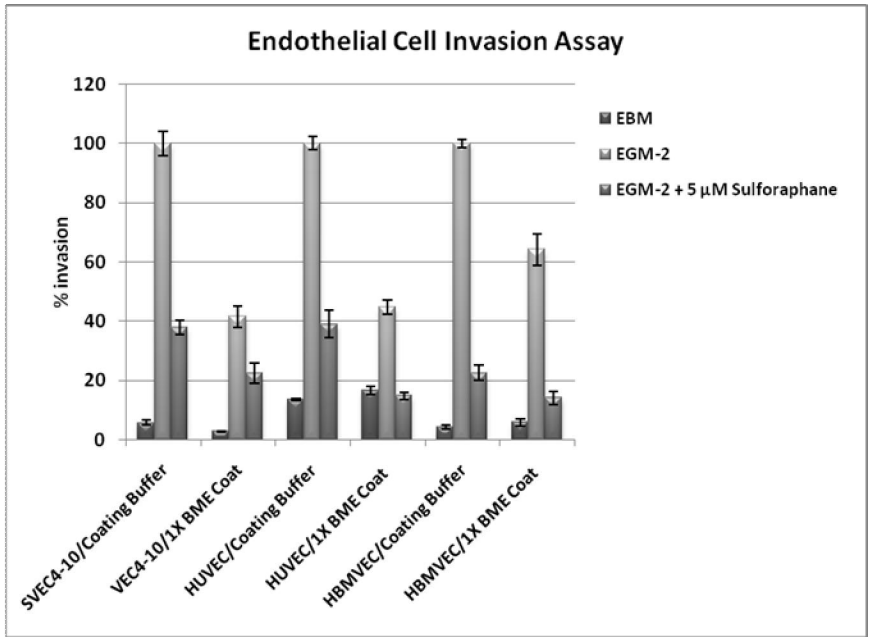

Figure 3. Relative quantification (as a percent of the control) of the ability of endothelial cell lines (SVEC4-10, HUVEC and HBMVEC) to cross a barrier consisting of an 8 micron polyester filter uncoated (Coating Buffer) or coated with 1 X BME (basement membrane extract) over a 24 hours period in response to FBS, VEGF, bFGF and other growth factors containing in the EGM-2, in the presence or absence of 5 µM Sulforaphane. Y axis represent percent invasion relative to migration.

IX. Troubleshooting

Problem	Cause	Solution
No signal	Cells did not penetrate barrier/Barrier may not be permissive enough for cell type	Use more diluted coating solution.
	Cell type may be noninva-sive or chemoattractant may be insufficient.	Select for more invasive subpopulations by subculturing/ Optimize chemoattractant concentrations using a dilution series
	Number of cells is not enough to degrade matrix	Increase number of cells or increase time of the assay
	The FBS used lacks the appropriate chemo-attractant at the expected concentration	Screening of FBS lots may be necessary for optimal results.
	Cells may have died as a result of treatment/toxic test agent was used.	Test cells for viability in treatment regimen.
High background	Insufficient washing - agents in media, FBS, and/or chemoattractants may react with Calcein-AM.	Re-assay, and make sure to wash well.
	Contamination - proteases released by bacteria or mold may degrade the BME and activate Calcein-AM.	Start a new culture from seed stocks, and re-assay. If seed stock is contaminated, then it may be prudent to get new cells.
Well to well variability	Inconsistent pipetting	Calibrate pipettors, and monitor pipet tips for air bubbles.
	Membrane punctured with pipette tips	Disregard data from wells that are punctured; re-assay if necessary.

X. References

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XI. Related products available.

Catalog#	Description	Size
3470-096-K	In Vitro Angiogenesis Assay Kit, Tube Formation	96 tests
3455-024-K	Cultrex® 24 Well BME Cell Invasion Assay	24 inserts
3455-096-K	Cultrex® 96 Well BME Cell Invasion Assay	96 samples
3460-024-K	CultreCoat® 24 Well BME-Coated Cell Invasion Assay	24 inserts
3465-024-K	Cultrex® 24 Well Cell Migration Assay	24 samples
3465-096-K	Cultrex® 96 Well Cell Migration Assay	96 samples
3456-096-K	Cultrex® 96 well 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

Accessories:

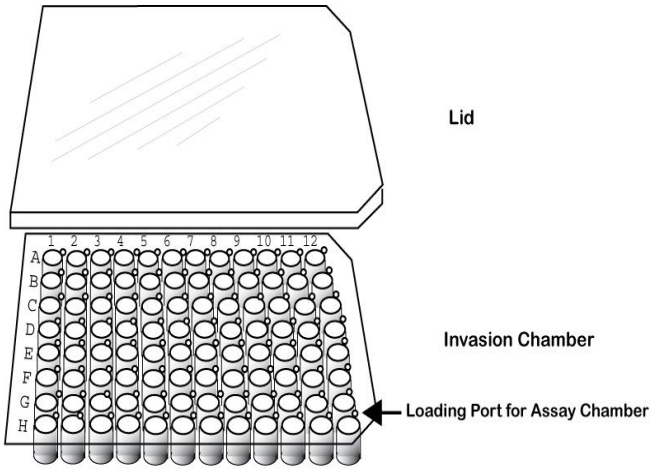
Catalog#	Description	Size
3400-010-01	Cultrex® Mouse Laminin I	1 mg
3440-100-01	Cultrex® Rat Collagen I	100 mg
3442-050-01	Cultrex® Bovine Collagen I	50 mg
3410-010-01	Cultrex® Mouse Collagen IV	1 mg
3430-005-02	Cultrex® BME with phenol red, PathClear®	5 ml
3431-005-02	Cultrex® BME with phenol red, reduced growth factor, PathClear®	5 ml
3432-005-02	Cultrex® BME no phenol red, PathClear®	5 ml
3433-005-02	Cultrex® BME no phenol red, reduced growth factor, PathClear®	5 ml
3430-005-01	Cultrex® BME with Phenol Red	5 ml
3432-005-01	Cultrex® BME; no Phenol Red	5 ml
3431-005-01	Cultrex® BME with Phenol Red; Reduced Growth Factors	5 ml
3433-005-01	Cultrex® BME; no Phenol Red; Reduced Growth Factors	5 ml
3416-001-01	Cultrex® Bovine Fibronectin	1 mg
3417-001-01	Cultrex® Bovine Vitronectin	50 µg
3438-100-01	Cultrex® Poly-L-Lysine	100 ml
3443-050-03	Cultrex® Murine VEGF	1 µg
3443-050-02	Cultrex® Human FGF-2	5 µg
3443-050-01	Cultrex® Human EGF	50 µg
3443-050-04	Cultrex® Human β-NGF	2 µg
3437-100-K	Cultrex® Cell Staining Kit	100 ml
3439-100-01	Cultrex® Cell Recovery Solution	100 ml
3450-048-05	CellSperse™	15 ml

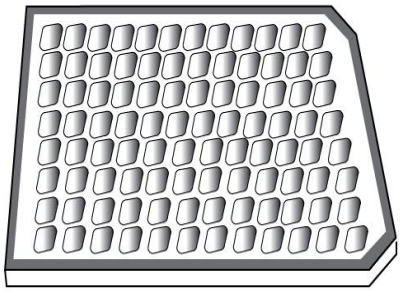
XII. Appendix

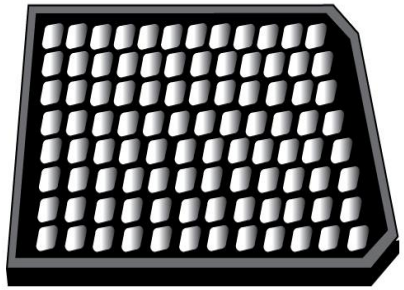
Appendix A. Reagent and Buffer Composition

1. **5X BME Solution**
   BME is provided at 10 mg/ml, derived from EHS tumor. Avoid freeze-thaws.
2. **10X Coating Buffer**
   Proprietary buffer optimized for coating BME to polyester membrane.
3. **25X Cell Wash Buffer**
   PBS buffer for washing cells (250 mM Potassium Phosphate (pH 7.4), 3.625 M NaCl)
4. **10X Cell Dissociation Solution**
   Proprietary formulation containing sodium citrate, EDTA, and glycerol.
5. **Calcein-AM**
   A non-fluorescent, hydrophobic compound that easily permeates intact, live cells. Once in the cell, Calcein-AM is hydrolyzed by intracellular esterases to produce Calcein, a hydrophilic, strongly fluorescent compound that is retained in the cell cytoplasm.

6. **Sulforaphane, 10mM**
   Sulforaphane [1-isothiocyanato-(4R)-methlysulfinyl)-butane]
7. **Cell Invasion Chambers**
   96 Well Boyden Chamber, 8.0 µm PET membrane, clear observation plate; black receiver plate compatible with 96 well fluorescent plate reader.




Culture Chamber


Assay Chamber