CULTREX® Instructions

For Research Use Only. Not For Use In Diagnostic Procedures

Cultrex® 3-D Spheroid Colorimetric Proliferation/Viability Assay

Reagent kit for investigating spheroid cell proliferation and/or viability

96 samples

Catalog #: 3511-096-K

Cultrex® 3-D Spheroid Colorimetric Proliferation/Viability Assay

Catalog # 3511-096-K

Table of Contents

I. Quick Reference Procedure for Trevigen's 3-D Spheroid Colorimetric Proliferation/Viability Assay:

Read through the complete Instructions for Use prior to using this kit. Determine the optimal seeding density for each cell line used. In general, 3,000 cells per well is a good starting point.

1. Culture cells per manufacturer's recommendation; adherent cells should be cultured to no more than 80% confluence.
2. Thaw 10X Spheroid Formation ECM on ice for two hours or overnight in a 4°C refrigerator.
3. Harvest cells (section VII.A) and resuspend in 1X Spheroid Formation ECM (section VI.1).
4. Aliquot 50 µl of cell suspension per well of the 3D Culture Qualified 96 Well Spheroid Formation Plate. 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. Prepare spheroid treatments in complete culture medium, and add treatments to spheroids.
8. Incubate at 37 °C in a tissue culture incubator for desired treatment period. If spheroid growth and/or inhibition are to be evaluated using image analysis, photograph the spheroid in each well every 24 hours using the 4X objective.
9. At the end of the treatment period, add one tenth volume (10 µl per 100 µl) of MTT Reagent per well, and transfer the plate back to the 37 °C cell culture incubator for 24 hours.
10. Warm Detergent Reagent at 37 °C for 5 minutes, and add an equivalent volume (100 µl for 100 µl) of Detergent Reagent per well. Transfer the plate back to the 37 °C cell culture incubator for 24 hours.
11. Read absorbance at 570 nm.

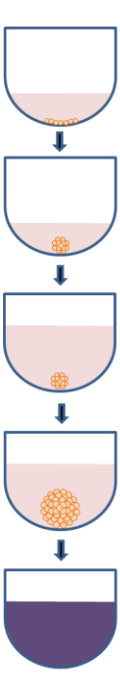
II. Background

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. This process can be monitored in real-time and label-free using image analysis software to measure spheroid area, and the kit is supplied with the colorimetric cell viability reagent MTT for quantitative end point analysis. The MTT tetrazolium ring is cleaved by mitochondrial dehydrogenases yielding purple formazan crystals which are subsequently solubilized for quantitation using Detergent Reagent[11].

The 3-D Spheroid Colorimetric Proliferation/Viability Assay provides a useful tool for modeling tumor response *in vitro*. The kit utilizes a 3-D Culture Qualified 96 Well Spheroid Formation Plate alongside a specialized Spheroid Formation ECM to drive aggregation and/or spheroid formation of cells. Upon completion of

spheroid formation, the spheroid may be treated with pharmacological agents to evaluate tumor viability after drug treatment. Tumor spheroid expansion is visualized microscopically and can be quantitated through image analysis software for real-time and label free evaluation. At the conclusion of the assay, cell viability may be assessed by absorbance using MTT. The 3-D Spheroid Colorimetric Proliferation/Viability Assay offers an *in vitro*, standardized, three-dimensional, high content format for inducing multicellular tumor spheroid (MCTS) formation and quantitating cell viability within the spheroids in response to pharmacological treatment.



STEP	TIME
Resuspend cells in Spheroid Formation ECM and add to 96 Well Spheroid Formation Plate.	1-2 hours
Cells assemble into compact spheroids.	3 days
Add medium containing cell proliferation modulating compounds.	1 hour
Cells proliferate and spheroids expand over time.	3-6 days
Cells reduce MTT to a purple formazan to quantify cell number.	2 days
	Total Time: 8-11 days

Figure 1. Steps comprising the 3-D Spheroid Colorimetric Proliferation/Viability Assay.

III. Precautions and Limitations

1. For Research Use Only. Not for use in diagnostic procedures.
2. The physical, chemical, and toxicological properties of these products 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. The **CULTREX® 3-D Spheroid Colorimetric Proliferation/Viability Assay** 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.

4. MTT Reagent (Cat# 4890-25-01) contains less than 1% (w/v) MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl-tetrazolium bromide (CAS # 298-93-1). MTT is toxic and may cause heritable genetic defects. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious. Call a physician.
5. Detergent Solution (Cat# 4890-25-02) contains SDS which is an irritant. In case of contact, immediately flush eyes or skin with copious amounts of water. If swallowed, wash out mouth with water provided person is conscious.

IV. Materials Supplied

Component	Quantity	Storage	Catalog #
10X Spheroid Formation ECM	600 µl	-80˚C	3500-096-01
3D Culture Qualified 96 Well Spheroid Formation Plate	Each	Room Temp	3500-096-02
MTT Reagent	2.5 ml	4˚C	3511-096-01
Detergent Reagent	25 ml	4˚C	3511-096-02

V. Materials/Equipment Required But Not Supplied

Equipment

1. 1 - 20 µl pipettor, 20 - 200 µl pipettor, and 200 - 1000 µl pipettor
2. Laminar flow hood or clean room
3. 37˚C CO_2 incubator
4. Low speed swinging bucket 4°C centrifuge and tubes for cell harvesting
5. Hemocytometer or other means to count cells
6. -80˚C storage
7. Ice bucket
8. Standard light microscope (or inverted)
9. Pipette aid
10. Timer
11. Inverted bright field microscope with 4X objective and digital camera
12. Computer
13. Graphing software, such as Microsoft® Excel®.
14. Image analysis software, such as ImageJ.
15. Colorimetric plate reader

Reagents

1. Cell line(s) of interest.
2. Cell harvesting buffer; EDTA, trypsin, or other cell detachment buffer.
3. Tissue culture growth media, as recommended by cell supplier.
4. Pharmacological agents for addition to culture medium, if necessary.
5. Sterile PBS or HBSS to wash cells.

6. Trypan blue or equivalent viability stain

Disposables

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

VI. Reagent Preparation

1. 10X Spheroid Formation ECM

10X Spheroid Formation ECM should be thawed on ice at 4 °C and diluted with Tissue Culture Growth Medium chilled to 4 °C; pipet up and down with a serological pipet to mix. Cells are resuspended in 1X Spheroid Formation ECM, and 50 µl of cell suspension is added to each well of the 3D Culture Qualified 96 Well Spheroid Formation Plate. See Table 1 for recommended dilution schedules.

2. MTT Reagent

The MTT Reagent is supplied ready for use. The MTT Reagent is stable at 4 °C provided there is no contamination. Care should be taken not to contaminate the MTT reagent with cell culture medium during pipetting. It is recommended that the appropriate volume required for each experiment is aliquotted and placed into a separate clean tube under sterile conditions and the stock bottle is returned to 4 °C in the dark. If the MTT Reagent is blue-green in color do not use and refer to the Troubleshooting Guide.

3. Tissue Culture Medium with Proliferation/Viability Modulating Compounds

Add 2X concentration of Proliferation/Viability Modulating compounds within the tissue culture medium to compensate for changes in total volume due to the spheroid formation ECM. Tissue culture medium should be at 37 ˚C when added.

4. Detergent Reagent

The Detergent Reagent is supplied ready for use. Before use, warm the bottle for 5 minutes at 37 ˚C then invert gently while mixing to avoid frothing.

VII. Assay Protocol

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

A. Cell Harvesting

Culture cells per manufacturer's recommendation. The following procedure is suggested and may need to be optimized to suit the cell type(s) being studied.

1. Cells need to be healthy and proliferating prior to use in the assay. Cells should be passaged 2 or 3 times and evaluated for cell viability by trypan blue or equivalent assay. Do not start the assay until cell viability is greater than 90%.
2. Each well requires approximately 2,000 - 5,000 cells, and 25 and 75 cm^2 flasks yield at least 1 x 10^6 and 3 x 10^6 cells, respectively. Plan accordingly.
3. Prior to harvest, visually inspect cells, and record cell health, relative number, and morphology.
4. 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.
5. Harvest cells. For 25 cm^2 flask or 75 cm^2 flask, add 1 ml or 2 ml, respectively, of Cell Harvesting Buffer (see Materials Required But Not Supplied), and incubate at 37˚C for 5 to 15 minutes until cells have dissociated from bottom of flask.
6. Transfer cells to a 15 ml conical tube, and add 5 ml of cell culture medium.
7. Centrifuge cells at 200 x *g* for 3 minutes to pellet cells, remove medium, and resuspend cells in 2 ml of cell culture medium. Cells may need to be gently pipetted up and down with serological pipette to resuspend cells and break up cell aggregates. Visually inspect cells to verify formation of a single cell suspension, no aggregates prior to counting.
8. Count cells and evaluate cell viability by trypan blue exclusion or equivalent test. Do not start the assay if cell viability is less than 90%.
9. Dilute to 1 x 10^6 cells per ml in cell culture medium.

B. 3-D Spheroid Colorimetric Proliferation/Viability Assay

1. Assay Preparation – Prior to Day 0

a. Establish assay parameters and appropriate controls. Appropriate controls include samples with and without proliferation/viability modulating agents, as well as samples without cells for background determination.

b. Determine optimal seeding density for each cell line used. In general, 3,000 cells per well is a good starting point. Optimal seeding density can be evaluated by analyzing the size of spheroid formation using the Spheroid Formation protocol (VII.B.2.). Serial dilutions of cells may be placed in each well to create a standard curve; this may be useful in evaluating spheroid size and linearity of the MTT absorbance curve (VIII.3.).

c. Culture cells per manufacturer's recommendation; adherent cells should be cultured to no greater than 80% confluence.

d. Thaw 10X Spheroid Formation ECM on ice overnight in a 4°C refrigerator.

2. Spheroid Formation – Day 0

a. Harvest and count cells, as directed in section VII. A.

b. Prepare a single cell suspension in 1X Spheroid Formation ECM. See section VI.1. for reagent preparation.

Table 1 Dilution Schedules (10% overage for multiwell dispensing):

Reagent	1 Well	24 Wells	96 Wells
10X Spheroid Formation ECM (4 °C)	5 µl	132 µl	528 µl
Tissue Culture Growth Medium (4 °C)	45-X µl	1188-X µl	4752-X µl
Cells	X µl	X µl	X µl
Total	50 µl	1320 µl	5280 µl

c. Dispense 50 µl of the single cell suspension in 1X Spheroid Formation ECM per well of the 3D Culture Qualified 96 Well Spheroid Formation Plate. Preserve unused wells for subsequent experiments by applying the strip seals that are included with each plate, if needed.

d. Centrifuge at 200 x *g* for 3 minutes at room temperature in a swinging bucket rotor.

e. Incubate at 37 °C in a tissue culture incubator for 72 hours to promote spheroid formation.

3. 3D Culture Spheroid Treatment – Day 3

a. Add 50 µl of warm (37 ˚C) cell culture medium containing proliferation/viability modulating compounds, if applicable. See section V.3 for reagent preparation.

b. Incubate the plate 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. Adjust the lighting and focus to provide the most contrast between the 3D structure and background. The use of fluorescence microscopy for cells that express fluorescent protein or have a fluorescent label may also improve contrast and subsequent analysis. Debris may transfer to the bottom of the 96 well plate during handling; wiping the bottom of the wells with lens paper prior to photographing may improve clarity. The assay may be conducted longer than 6 days if desired; however, changing cell culture medium may be required to maintain cell viability.

c. Analyze images using image analysis software to measure changes in the area of the structures to determine the extent of 3-D culture spheroid expansion for each sample.

4. **Image Analysis**

Note: Images may be analyzed using free software such as ImageJ (http://rsb.info.nih.gov/ij/); see below. Other image analysis software may be configured to make the same measurements; please consult your software supplier regarding capabilities and instructions.

a. Photograph image of known size (eg. 1 mm) using 4X objective, and measure pixels in imageJ.
   i. Open image. Go to File/Open, and select image.
   ii. Select line tool.
   iii. Draw a line the length of the object.
   iv. Go to Analyze/Measure, and record pixel number.
   v. Calculate the number of pixels in each mm (eg. 600 pixels/mm).

   Record result _____pixels/mm

b. Set scale; this will need to be done each time ImageJ is opened.
   i. Go to Analyze/Set Scale.
   ii. For “Distance in pixels” input value from VII.B.4.v. (above).
   iii. For “Know distance” input “1,000”.
   iv. For “Unit of Length” input “um”.
   v. Check “Global”, and select “OK”.

c. Analyze spheroid image.
   i. Open image. Go to File/Open, and select image.
   ii. Convert to an 8 bit image: Go to Image/Type/8 bit (check).
   iii. Adjust image threshold. Go to Image/Adjust/Threshold (check). The program will distinguish between dark and light pixels, and the threshold may be adjusted on the histogram using the sliding scale. Similar settings will work for photographs taken under the same conditions. Areas of dark pixels will be overlaid with red. Adjust the threshold so that only the spheroid image is overlaid.
   iv. Select spheroid. Select the circle tool and surround the image. This will eliminate any dark pixels outside of the spheroid from the measure.
   v. Go to Analyze/Measure. This will measure the area of the spheroid structure (μm^2) in a table. Multiple measurements may be made in one table. Save table.
   vi. Convert the table to an Excel worksheet. Open with Excel and “Save As” an .xls or .xlsx file.

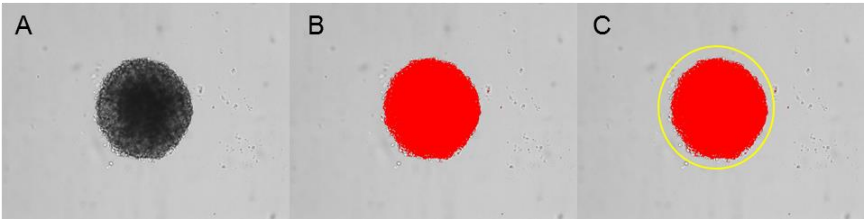


Figure 2. Process for analysis of spheroid expansion. A) Capture image and convert to 8 bit; B) set threshold to capture the total structure; and C) select structure to calculate total area.

5. Colorimetric analysis

a. Add one tenth volume (10 µl per 100 µl) of MTT Reagent per well and transfer the plate back to the 37 °C cell culture incubator for 24 hours. See section V.3 for reagent preparation.

b. After 24 hours, warm Detergent Reagent to 37 °C, and add an equal volume (100 µl per 100 µl) of Detergent Reagent. Transfer the plate back to the 37 °C cell culture incubator for 24 hours to solubilize cells and MTT formazan crystals.

c. Read absorbance at 570 nm. If substantial cell debris is present after incubation, background values may be evaluated at 690 nm and deducted for each sample.

d. Graph data.

VIII. Data Interpretation

1. The **CULTREX® 3-D Spheroid Colorimetric Proliferation/Viability Assay** provides morphological and quantitative analysis of spheroid cell proliferation/viability. Spheroids expand in size as cells proliferate, and this results in changes in surface area that occur over time for proliferating cell lines, see figure 3. These changes in surface area can be measured and used to compare relative proliferation of different cell lines or cell lines subjected to genetic manipulation.

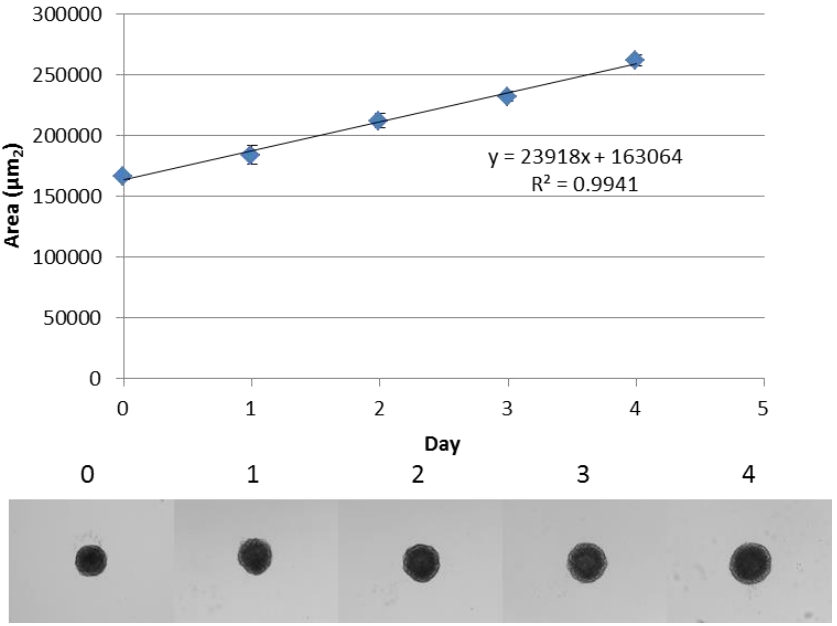
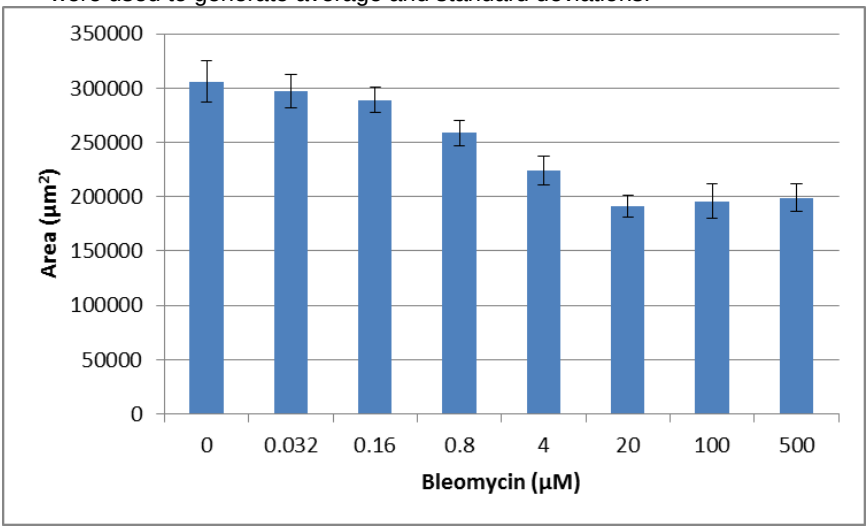


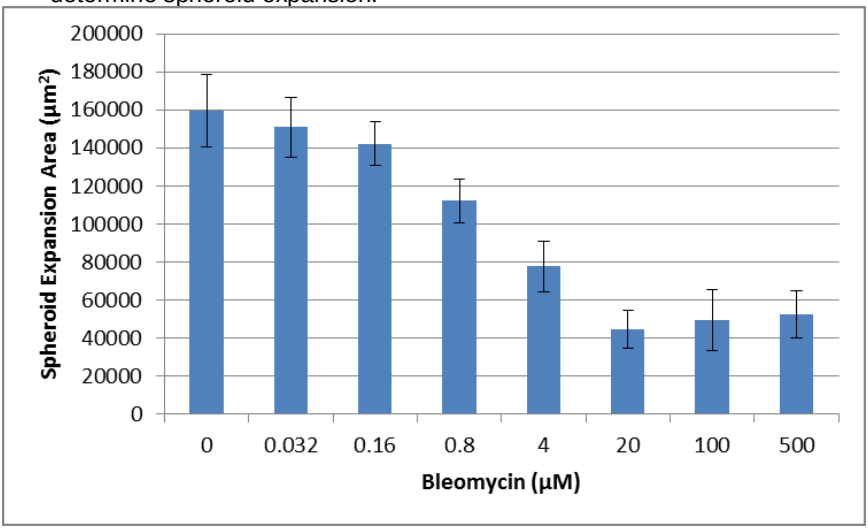
Figure 3. Spheroid growth of MDA-MB-231 breast cancer cells. Cells were seeded at 3,000 cells/well in the presence of spheroid formation ECM and incubated for 72 hours at 37 °C, 5% CO_2 to induce spheroid formation. At that time, 50 µl of complete medium was added to each well, and spheroids were incubated at 37 °C, 5% CO_2. Spheroids were photographed every 24 hours, and images were analyzed using ImageJ software.

2. The changes in area can also be used to evaluate the effect of pharmacological compounds on spheroid growth. Once optimal assay conditions have been established, this can be evaluated as an endpoint assay. The following assay was conducted using MDA-MB-231 cells treated with varying concentrations of the inhibitor Bleomycin.

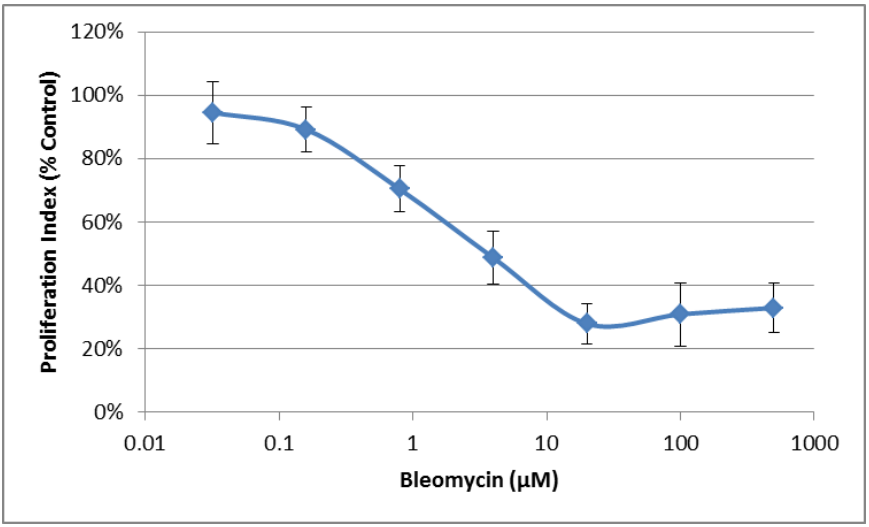
a. First the areas were calculated for each condition, and these values were used to generate average and standard deviations:



b. Next the area for each spheroid prior to treatment was subtracted to determine spheroid expansion.

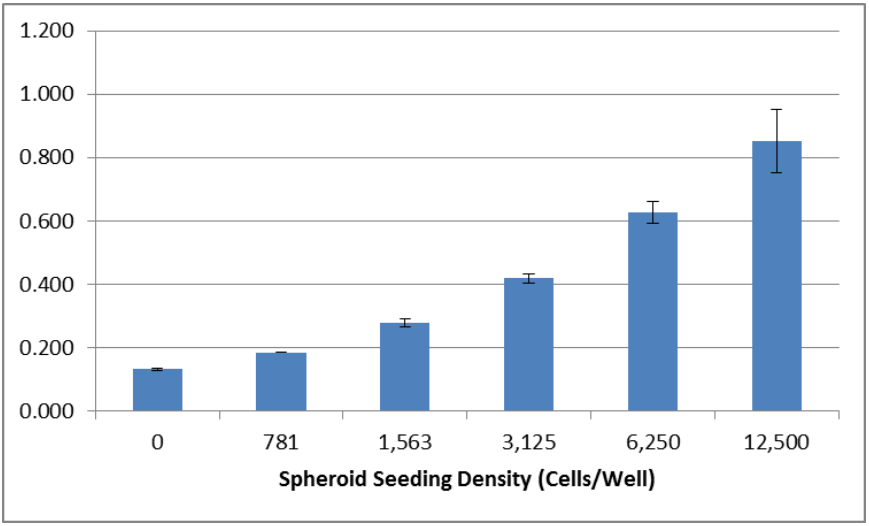


c. Finally, the resulting areas were divided by the area obtained for the no inhibitor control, providing the proliferation index as a percentage of control:

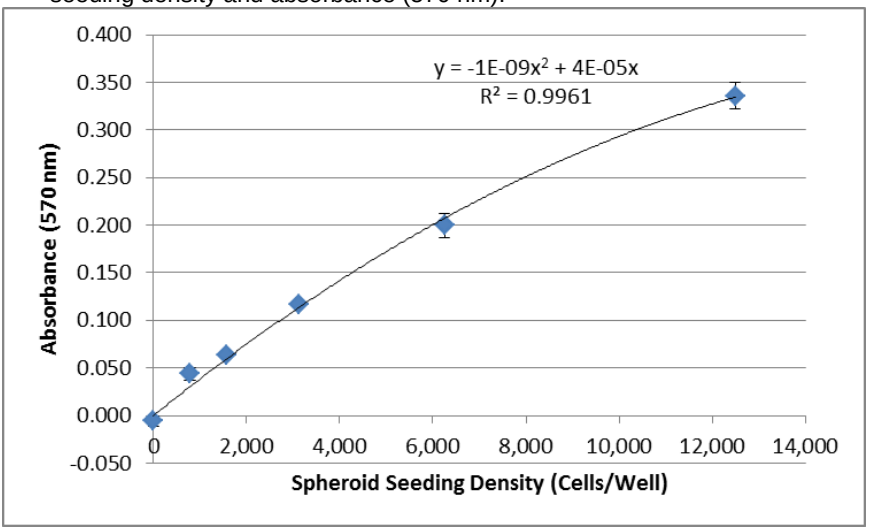


3. Colorimetric endpoint analysis using The MTT Reagent can be used to generate a standard curve based on cell seeding density. The following assay was conducted using MDA-MB-231 cells serially diluted from 12,500 cells/well to 781 cells/well on Day 0 and evaluated on Day 3.

a. First the absorbance was measured at 570 nm for each well, and the average and standard deviations were calculated for each sample using Excel:

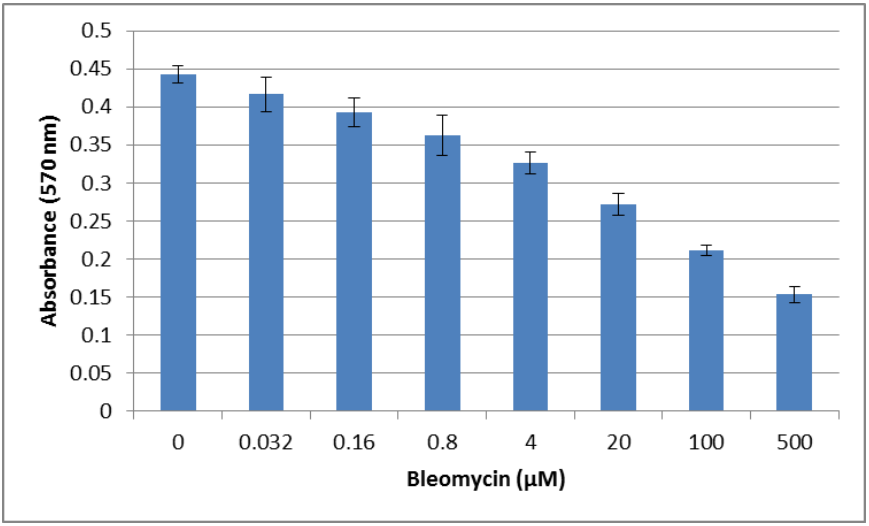


b. Next the background was subtracted from each sample, and the data was plotted with a trendline to evaluate the relationship between cell seeding density and absorbance (570 nm):

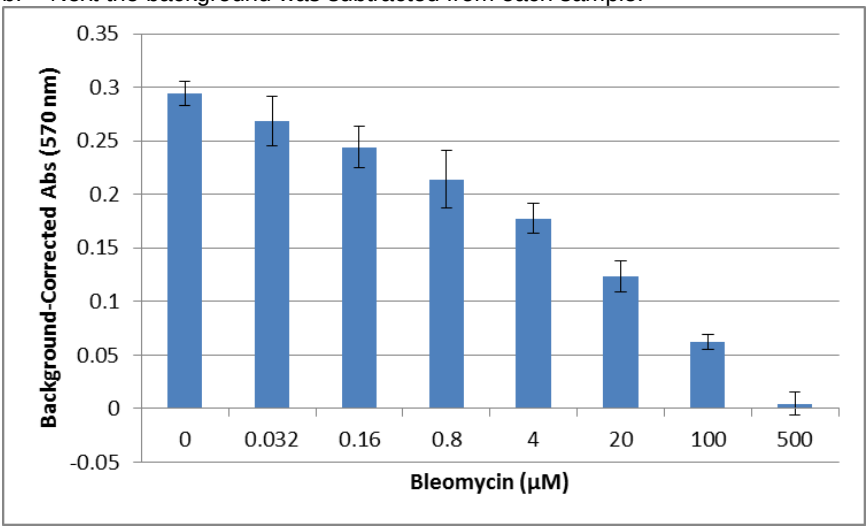


4. Colorimetric endpoint analysis using The MTT Reagent can also be used to evaluate the effect of pharmacological compounds on cell viability. The following assay was conducted using MDA-MB-231 cells treated with varying concentrations of the inhibitor Bleomycin.

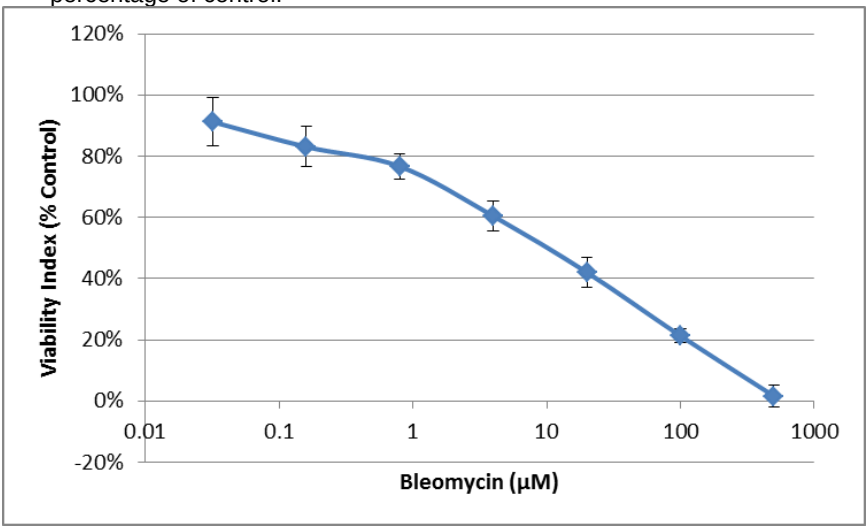
a. First the absorbance at 570 nm was measured for each well, and the average and standard deviations were calculated for each sample using Excel:



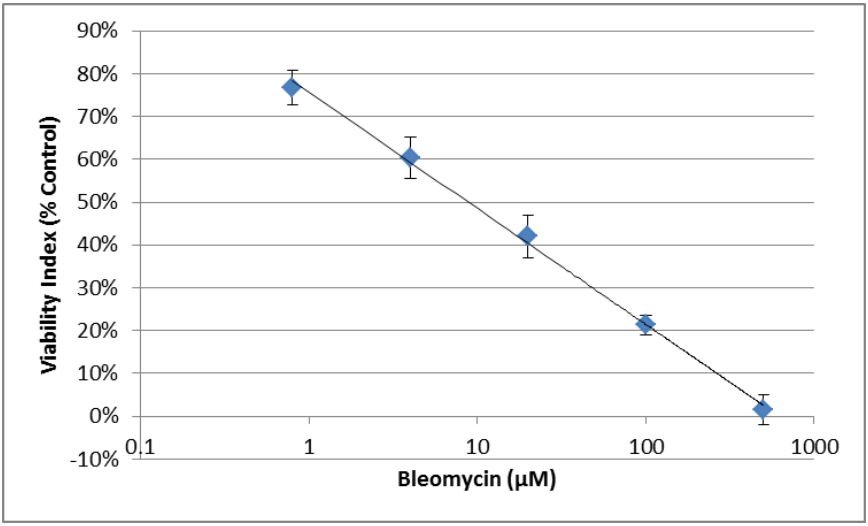
b. Next the background was subtracted from each sample:



c. Finally, the background-corrected absorbance was divided by the value obtained for the no inhibitor control, providing the viability index as a percentage of control:



d. Using the linear portion of the inhibition curve, the IC50 may be estimated:



IC50 ~ 15 µM Bleomycin for MDA-MB-231 spheroids.

IX. Troubleshooting

Troubleshooting Guide		
Problem	Cause	Solution
Cells do not form spheroids.	Cells are not healthy.	Assess cell doubling rates and check cell viability prior to spheroid formation.
	The cell line does not form spheroids.	While many cancer cell lines of epithelial origin have been shown to form spheroids, there may be exceptions. The kit may not be compatible with this cell line.
MTT Reagent is blue/green.	Contamination with a reducing agent or cell/bacterial contamination.	Discard, remove aliquots of new MTT reagent using sterile procedure.
	Excessive exposure to light.	Store solution in the dark at 4 ^{o}C.

Problem	Cause	Solution
Blanks (media only) give high absorbance readings.	The media is contaminated with cells/bacteria/yeast (visible under microscope).	Discard. Check media solution before plating. Use sterile technique for cell plating in biological hood. Use sterile 96 well plate.
	The media contains ascorbic acid.	Find alternative medium if possible. Incubate plate in the dark.
Absorbance readings are too low.	Cell number per well is too low.	Increase cell density at plating.
	Incubation time for reduction of MTT intracellularly too short. No purple color in cells visible when viewed under microscope.	Increase incubation time with MTT reagent until purple color evident inside cells when viewed under microscope.
	Incubation time for solubilization of formazan dye too short (intact cells with intracellular dye visible when viewed under microscope).	Increase incubation time with Detergent Reagent. View under microscope to ensure no crystals remain out of solution.
	Cells not proliferating due to improper culture conditions.	Check culture conditions (medium, temperature, humidity, CO_2 etc.) are appropriate. View cells periodically to check condition.

X. 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.
11. Ho, W.Y., et al., *Development of Multicellular Tumor Spheroid (MCTS) Culture from Breast Cancer Cell and a High Throughput Screening Method Using the MTT Assay.* PLoS ONE, 2012. **7**(9): p. e44640.

XI. Appendix A: Reagent and Buffer Composition

1. 10X Spheroid Formation ECM

Proprietary mixture of extracellular matrix proteins derived from murine EHS sarcoma cells optimized for spheroid formation. It is qualified for spheroid formation and provided in DMEM with 10 μg/ml gentamycin. Avoid freeze-thaws.

2. 3D Culture Qualified 96 Well Spheroid Formation Plate

96 Well, round bottom plate with low adhesion surface to promote spheroid formation, provided with strip seals to preserve sterility of unused wells for subsequent experiments.

3. MTT Reagent

MTT Reagent contains less than 1% (w/v) MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyl-tetrazolium bromide (CAS # 298-93-1).

4. **Detergent Reagent**

Detergent Solution contains SDS (CAS # 151-21-3) which is an irritant.

XII. Related products available from Trevigen.

Catalog #	Description	Size
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™ Rat Collagen I	20 ml
3445-096-K	3D Culture BME Cell Proliferation Assay Kit	96 samples
3446-096-K	3D Culture Laminin-I Cell Proliferation Assay Kit	96 samples
3447-096-K	3D Culture Collagen I Cell Proliferation Assay Kit	96 samples
3448-096-K	3D Culture Cell Harvesting Kit	20 samples
3455-096-K	Cultrex® BME Cell Invasion 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
3500-096-K	Cultrex® 3-D Spheroid BME Cell Invasion Assay	96 samples
3510-096-K	Cultrex® 3-D Spheroid Fluorometric Proliferation/Viability Assay	96 samples
4892-010-K	Cultrex® Calcein-AM Cell Viability Kit	1000 samples
4890-025-K	TACS® MTT Cell Proliferation Assay	2500 samples
4891-025-K	TACS® XTT Cell Proliferation Assay	2500 samples
4822-96-K	HT TiterTACS™ Assay Kit	96 samples
4817-60-K	FlowTACS™ Apoptosis Detection Kit	60 samples
4830-01-K	TACS® Annexin V FITC Kit	100 samples
4835-01-K	TACS® Annexin V Biotin Kit	100 samples
6300-100-K	DePsipher™ Mitochondrial Potential Assay Kit	100 samples
6305-100-K	MitoShift™ Mitochondrial Potential Assay Kit	100 samples

The product accompanying this document is intended for research use only and is not intended for diagnostic purposes or for use in humans.

Trevigen, Inc.
8405 Helgerman Ct. Gaithersburg, MD 20877
Tel: 1-800-873-8443 • 301-216-2800
Fax: 301-560-4973
e-mail: info@trevigen.com
www.trevigen.com

Please Recycle