Materials List for:

Characterization of the Effects of Migrastatic Inhibitors on 3D Tumor Spheroid Invasion by High-resolution Confocal Microscopy

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Materials

Name	Company	Catalog Number	Comments
Collagen I, rat tail, 100 mg	Corning	354236	for glioma invasion assay; this is offered by many distributors/ manufacturers and will need to be determined for both the type of assay intended and cell lines used. For glioma cancer cell lines Collagen rat tail type 1 (e.g. Corning) is the preferred choice. Collagen should be stored at 4 °C, in the dark, until required. It is not advisable to mix collagen from different batches as this may affect the consistency of the polymerized collagen.
Coverslips	various	various	for microscopy imaging
DMEM powder	Sigma	D5648	needed at 5x concentration for collagen solution for glioma invasion assay; this may be purchased in powdered form, made up in double distilled water and, depending upon final composition of the growth medium, completed with any additives required. The complete 5x solution should be filtered through a syringe filter system (0.22 µm) before use.
Foetal calf serum	Sigma	F7524-500ML	needed for cell culture of glioma cell lines
Glass slides	various	various	for microscopy imaging
High glucose DMEM	Gibco	41965062	needed for cell culture of glioma cell lines
Inhibitor	Tocris	various	various - according to experimental design; inhibitors can be purchased from manufacturers such as Selleckchem and Tocris. These manufacturers offer detailed description of inhibitor characteristics, links to associated references and suggestion of working concentrations. As with all inhibitors, they may be potentially toxic and should be handled according to health and safety guidelines. Inhibitors are prepared as stock solutions as recommended by

			the manufacturer. As an example we used the migrastatic inhibitor MI-192 to demonstrate the use of such inhibitors. We have tested a range of migrastatic inhibitors in this way with comparable results.
Mountant	various	various	for microscopic imaging
NaOH (1 M)	various	various	NaOH can be either purchased at the required molarity or prepared from solid form. The prepared solution should be filter sterilized using a syringe filter system. One M NaOH is corrosive and care should be taken during solution preparation.
Paraformaldehyde	various	various	for fixing spheroids and cells; make up at 4%, caution health hazard, ensure that health and safety regulations are adhered to for collagen solution for invasion assay
Pastettes (graduated pipette, 3 mL)	various	various	for invasion assay, solution removal
PBS, sterile for tissue culture	Sigma	D1408-500ML	needed for cell culture of glioma cell lines and washes for staining
Pen/strep (antibiotics)	Sigma	P4333	needed for cell culture of glioma cell lines
Primary antibody, secondary antibody, DAPI, Phalloidin	various	various	there are many manufacturers for these reagents, for secondary labelled antibodies we recommend Alexa Fluor (Molecular Probes). Here we used for primary antibodies mouse anti-acetylated tubulin antibody (1/100, Abcam). For secondary antibodies we used 1/500 anti-mouse Alexa Fluor 488 conjugated antibody, Molecular Probes. For nuclear stain we used DAPI (many manufacturers) and the actin stain Phalloidin (many manufacturers) both used at recommended dilution of 1/500.
Sodium bicarbonate	Sigma	S5761	needed for collagen solution for glioma invasion assay at 5x concentration
Sodium pyruvate	Sigma	P5280	needed for collagen solution for glioma invasion assay at 5x concentration
Trypsin	Sigma	T4049	for trypsinisation
Ultra low attachment plates	Sigma/Nunc	CLS7007-24EA	for glioma invasion assay; a low adherent plate is required, with 96-well plates preferred to allow for large-scale screening of compounds under investigation. There are several low adherence plates commercially available; it is advisable to test a variety of plates for optimum spheroid generation. In our experience Costar Ultra Low Cluster with lid, round bottom, works best for the generation of spheroids from glioma cancer cells in terms of 100% spheroid formation and

			reproducibility. These plates were also successfully used for the generation of glioma spheroids from patient-derived material, bladder and ovarian cancer cells in our laboratory. In addition, stem or progenitor neurospheres can be used in these plates to facilitate the generation of standardized neurosphere-spheroids
Stripettes (serological pipettes, sterile, 5 mL and 10 mL)	various e.g. Costar	CLS4488-50; CLS4487-50	for tissue culture and collagen preparation
Various multichannel (50 - 250 µL) and single channel pipettes (10 µL, 50 µL, 200 µL 1 mL)	various	various	for cell and spheroid handling
Widefield microscopy	various	various	for observation of spheroid generation and spheroid imaging; here wide-field fluorescence images were captured using an EVOS FL cell imaging system (Thermo Fisher Scientific)
Zeiss LSM 880 CLSM equipped with a Plan Apochromat 63x 1.4 NA oil objective	Zeiss	quote from manufacturer	Confocal images were captured using a Zeiss LSM 880 CLSM equipped with a Plan Apochromat 63x 1.4 NA oil objective. Diode 405nm, 458/488/514 nm argon multiline and HeNe 594nm lasers were used to excite Phalloidin 594, Alexa Fluor 488, and DAPI respectively. For each image a single representative optical section were captured, with all settings, both pre- and post-image capture, maintained for comparative purposes. All images were subsequently processed using the associated Zen imaging software and Adobe Photoshop.