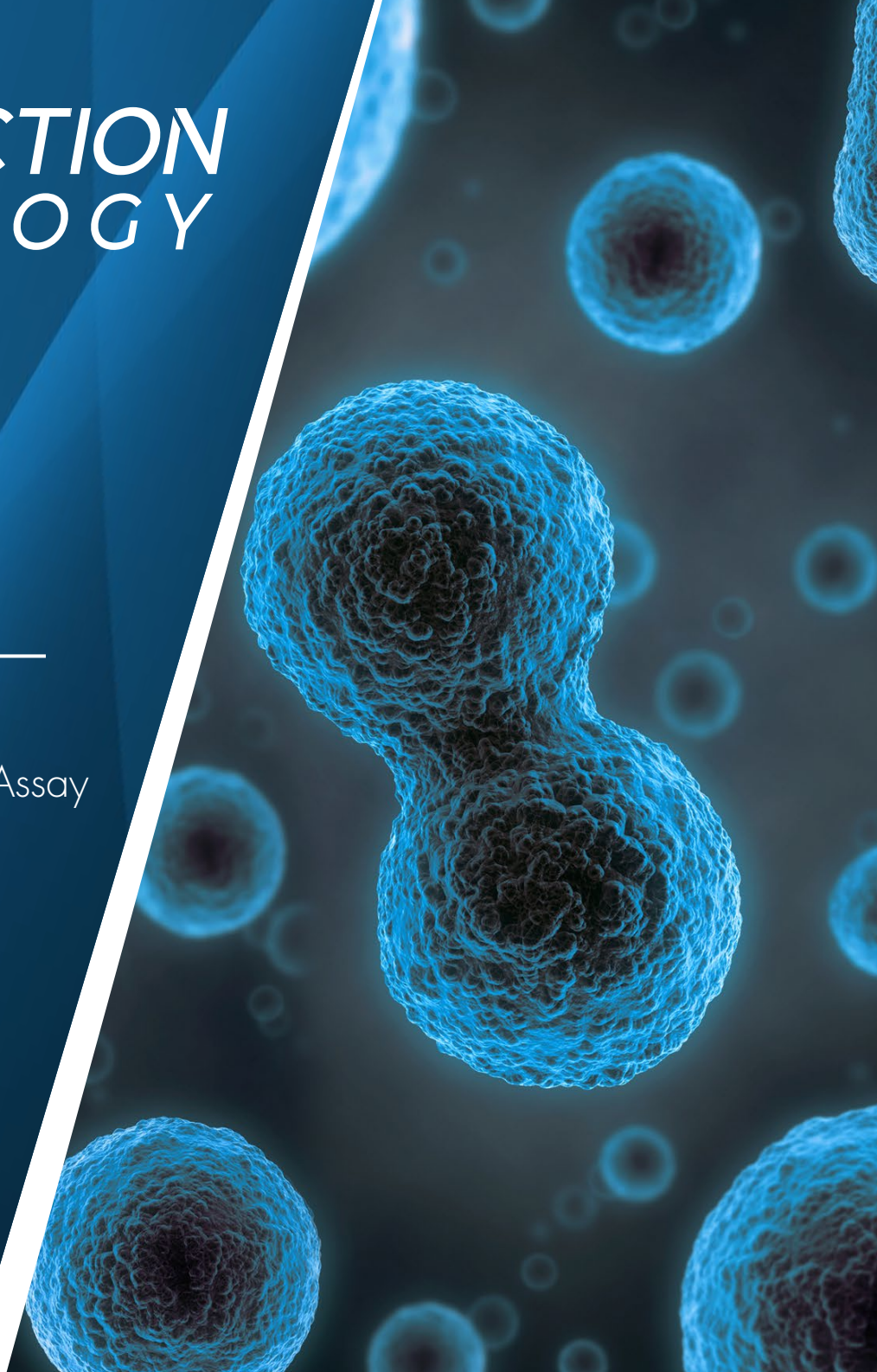
REACTION BIOLOGY

Cell-Based Oncology Assays

- Proliferation Assay
- 3D Tumor Spheroid Assay
- Soft Agar Assay
- Migration Assay
- Invasion Assay
- Angiogenesis Assay
- Cell Line Generation



Let’s discover together.

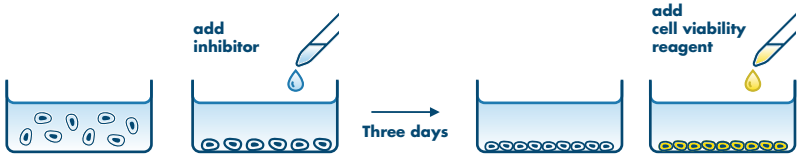
Cell-based Oncology Assays

Reaction Biology offers a variety of phenotypic assays for the discovery of anti-cancer drugs based on human tumor cell lines. All of our assays are high-throughput compatible and can be customized according to your specific requirements.

Cell Proliferation Assay

Cell proliferation assays are widely used and accepted in oncology drug discovery as an initial test for cytotoxicity and anti-proliferative potential of test compounds.

- Free choice option for the screening of any number of compounds on your choice of cell lines
- Our panel comprises 253 human tumor cell lines
- Readouts: CellTiter-Glo™ and other methods including ATPlite, CyQUANT, BrdU incorporation, MTT, Alamar Blue, and WST-8 readouts as well as real-time kinetics measured via IncuCyte
- Deliverable: IC50 values of cytotoxic or anti-proliferative capacity of compounds

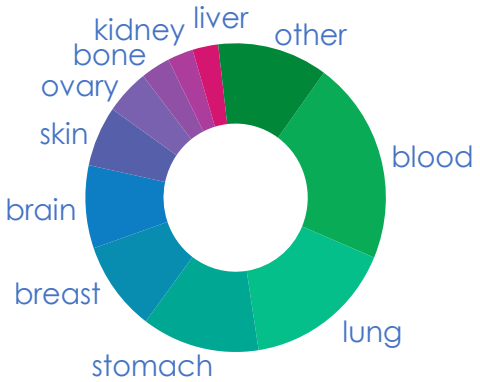


Assay procedure

Cells are grown in multi-well plates and incubated with compound for 72 hours. The number of viable cells is determined with CellTitre-Glo™ based on the amount of ATP present.

Cell lines available for the Cell Proliferation Assay

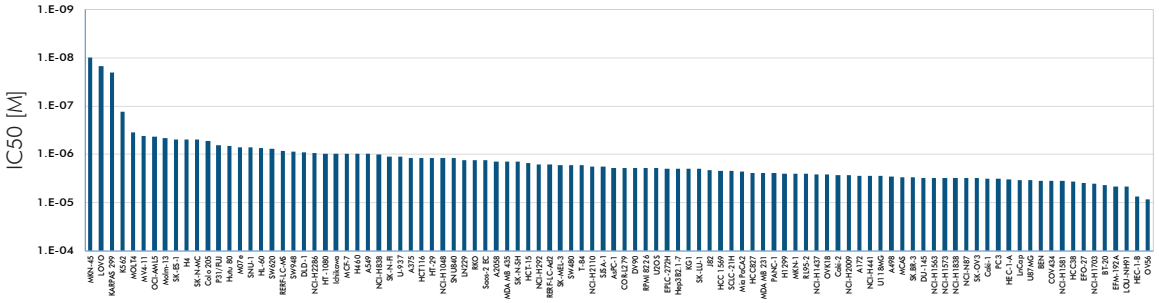
Tumor type	#	Tumor type	#
blood	54	liver	7
lung	41	pancreas	7
stomach	32	uterus	6
breast	24	prostate	5
brain	22	cervix	4
skin	16	muscle	3
ovary	12	adrenal gland	2
bone	8	fibrosarcoma	2
kidney	7	embryo	1



ProLiFiler – Cell panel screening

The ProLiFiler enables high-throughput analysis of the effects of test compounds on a panel of human cancer cell lines using the Cell Proliferation Assay.

- Performed bi-monthly, get your results in only 6 weeks. Huge cost-savings
- Available for single compound or combination therapy analysis
- Add our bioinformatics analysis to determine the mode of action of your compound and determine biomarkers based on the genetic signature of sensitive tumor cell lines



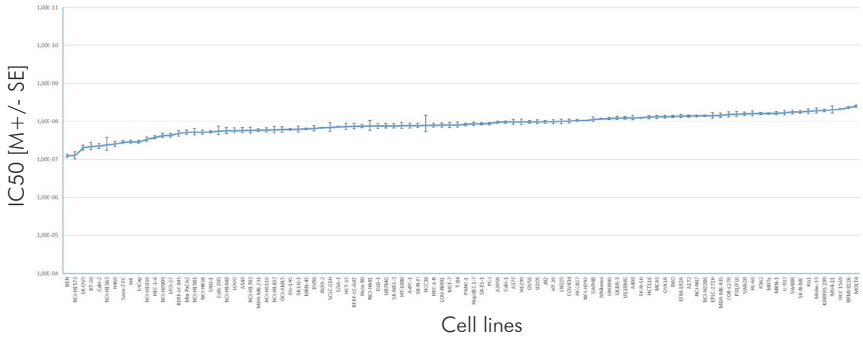
Example Crizotinib

Testing of standard of care compound Crizotinib in the ProLiFiler. The MET kinase inhibitor is active with low molarity only in some cell lines including MKN-45, LOVO and Karpas 299.

Reproducibility

Variation of reference compound, Bortezomib, across 4 independent ProLiFiler runs.

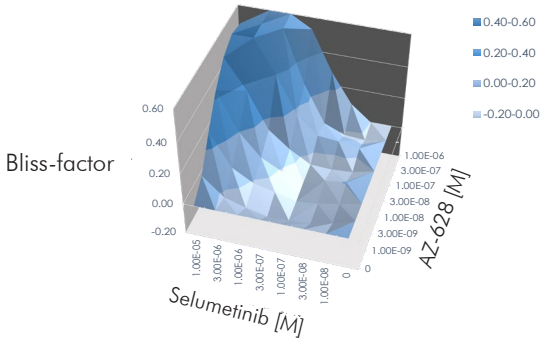
The semi-automated screening process produces highly reproducible data.



Combination Therapy with Cell Proliferation Assay

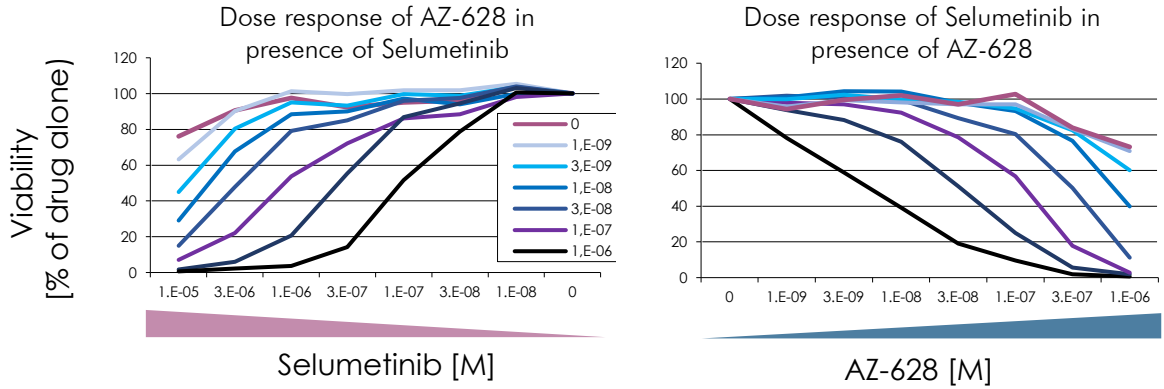
Combining drugs that affect different synergistically-acting targets have become a promising new strategy for cancer therapy.

- Combination drug testing can be performed with all of our cell-based oncology assays
- High-throughput option available
- Deliverable: IC_{50} values of cytotoxic or anti-proliferative capacity of compounds; Bliss-factor analysis of synergistic effects of two drugs



Bliss-Factor Analysis:

A Bliss-Factor matrix is used to compare the data obtained with the expected effects of the compounds alone, compared with the actual data obtained. A positive value between both numbers indicates synergism, represented as a hill on the plot.



Mutual synergy

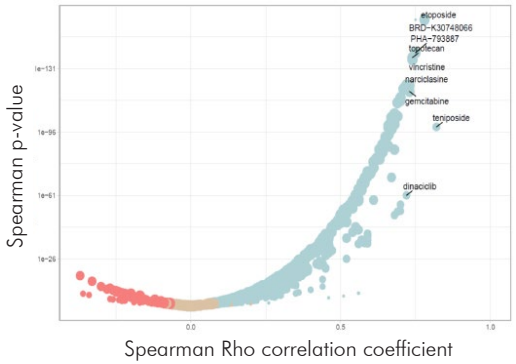
The addition of the second drug in increasing concentrations leads to reduced cell viability. In this example of mutual synergy, this effect can be seen for both drugs.

Bioinformatics Analysis of ProLiFiler Data

Partnered with 4HF Biotec GmbH, a bioinformatics firm specializing in cancer data mining, their comprehensive database of 1,800+ preclinical samples, 900+ reference drugs, and multiple cancer datasets are integrated into a single platform for visualization and statistical analysis.

MoA Finder tool:

To identify the mode of action (MoA) of your test compound based on the ProLiFiler cell panel screening results. We will compare the sensitivity profile of your drug candidate to 900+ MoA-known drugs.



Example: Doxorubicin

Correlation of drug sensitivity profile of doxorubicin to 732 reference drug profiles. Each dot represents one correlation. Reference drugs with a Spearman Rho correlation higher than 0.7 are named.

Biomarker Analysis tool:

Reveals the specific genomic sequence of tumor cell lines that are sensitive to your test drug. We correlate your test drug sensitivity profile (IC50 data of the ProLiFiler screen) with datasets of gene expression and genetic alterations (mutations, deletions) known for the tested tumor cell lines.

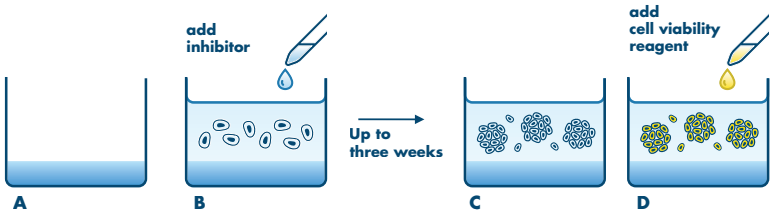
Gene symbol	Mutation counts in the Responder group	Mutation counts in the Non-Responder group	No Mutation counts in the Responder group	No Mutation counts in the Non-Responder group	Wilcoxon test p-value	Log2 of Wilcoxon test p-value	Mean absolute IC50 in non-mutated group	Mean absolute IC50 in mutated group
TP53	10	152	53	22	2.25E-17	55	11.6	27.5
KIAA1522	10	5	53	169	0.0002	12.3	23.2	11.6
FBXL8	6	1	57	173	0.0006	10.7	22.9	6.7

Example of the top three mutated genes correlated with nutlin-3a efficacy

Soft Agar Assay (Clonogenic Assay)

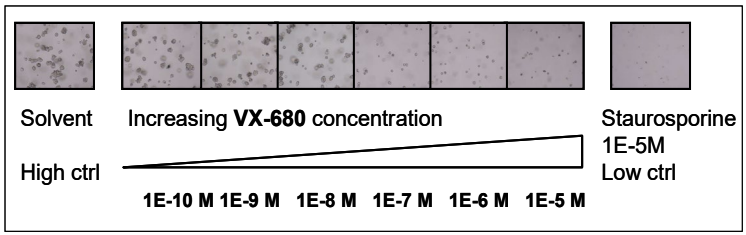
Anchorage-independent growth of cells is one of the hallmark characteristics of cellular transformation and uncontrolled cell growth. The soft agar allows the cells to grow to cell colonies independently of a solid surface.

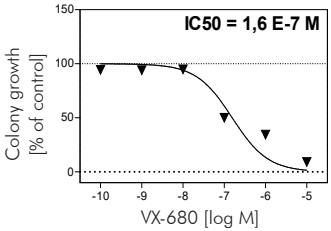
- Testing of anti-proliferative or cytotoxic efficacy of compounds while cells grow in an anchorage-independent way
- Up to three weeks of compound exposure
- Combination treatment possible
- Deliverable: IC_{50} values based on 8 concentrations of compound's potency to inhibit cell transformation



Soft Agar Assay procedure

Single-cell suspension in 0.4% soft agar is poured on a 0.6% soft agar layer. The inhibitor is added to the cells for incubation of up to three weeks. For quantification of live cells, cell viability indicator Resazurin is used.





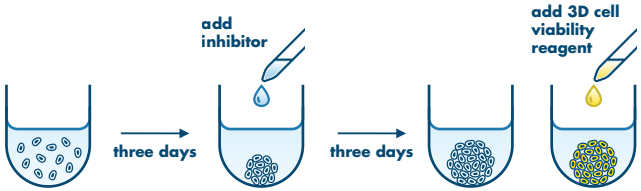
Example of Aurora B inhibition in A549 cells

A549 cancer cells were incubated with the Aurora B inhibitor VX-680 for 5 days in the Soft Agar Assay. VX-680 inhibited the formation of colonies with increasing concentrations.

3D Tumor Spheroid Assay

3D tumor spheroids recapitulate some of the complex processes that compounds face in an in vivo situation such as cellular barriers or metabolic changes due to gradients in oxygen and nutrient concentrations. These issues make screening with spheroids more relevant than with 2D cell culture.

- Mono-spheroid or co-culture spheroid testing
- Quantification of stroma and tumor cells
- High-throughput compatible
- Deliverables: EC_{50} values of cytotoxic effects of compounds

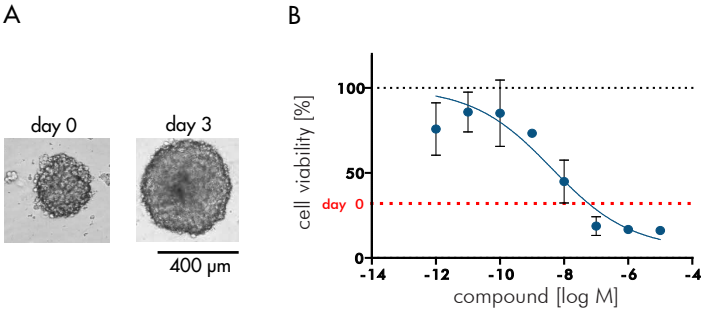


Assay principle

Tumor cells autonomously assemble to form spheroids in round-bottom 96-well plates. Compounds incubate for three days before readout of cell quantity with either cell viability reagent CellTitreGlo-3D™ or luciferase measurement.

Mono-Spheroid Assay

Spheroids in the mono-spheroid assay are composed of a single type of tumor cell. The readout for the quantification of live cells in the spheroid is performed via CellTitreGlo-3D™.



Example: U87MG mono-spheroids

A. Comparison of size of U87MG spheroids at day 0 and 3.

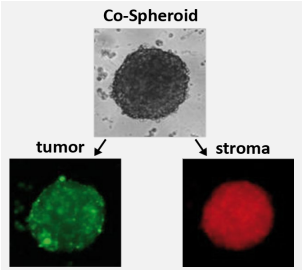
B. Dose-response of Trametinib treatment on U87MG mono-spheroids. Positive control (0% viability) is staurosporine treatment; negative control (100% viability) represents vehicle control. 'day 0' shows the number of viable cells present at the initiation of the experiment.

Cell lines available for mono-spheroid screening

Cell	Origin	Cell	Origin	Cell	Origin
J82	Bladder	CX-1	Colon	NCI-H441	Lung
SJSA-1	Bone	DLD-1		MCAS	Ovary
SKES-1		HCT-116		NCI-ADR	
LN229	Brain	HCT-15		RL95-2	
SK-NF-I		HT-29		MiaPaCa-2	Pancreas
SK-N-MC		SW620		LnCap	Prostate
U87MG		Hutu 80	Duodenum	A2058	Skin
MCF-7	Breast	HT-1080	Fibrosarcoma	A375	
MDA-MB-231		A498	Kidney	MDA-MB-435	
T47D		A427	Lung	Hs746T	Stomach
ZR-95-1		A549		NCI-N87	
HeLa	Cervix	H1299		SCH	
SiHa		H460			

Co-Culture Spheroid Assay

Firefly luciferase-expressing tumor cells and Renilla luciferase-expressing stroma cells are co-cultured to form spheroids. Both luciferases activities are measured at the end of the incubation to identify compound effects on both tumor and stromal cells.



Co-existence of tumor and stroma cells in spheroids

U87MG tumor cells are stained with Calcein, HS27A stroma cells are stained with RFP.

Stroma cells

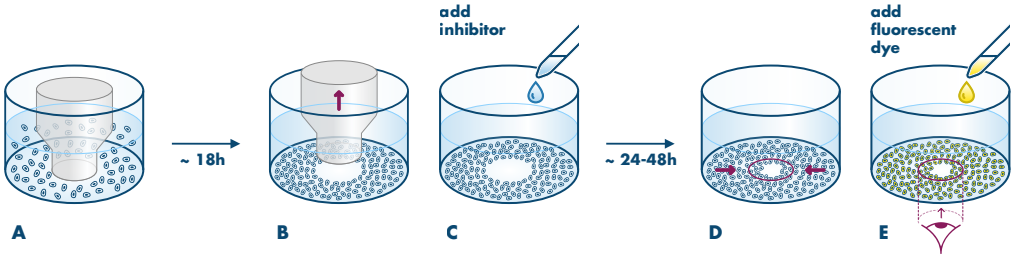
Tumor cells	HSS	HS27	NHDF
none	✓	✓	✓
A549	✓	✓	✓
DLD1	✓	✓	✓
HCT116	✓	✓	✓
LN229	✓	✓	✓
MCF7	✓	✓	✓
U87MG	✓	✓	✓

Tumor and stroma cell combinations available for co-culture spheroid screening.

Cell Migration Assay (Oris Assay)

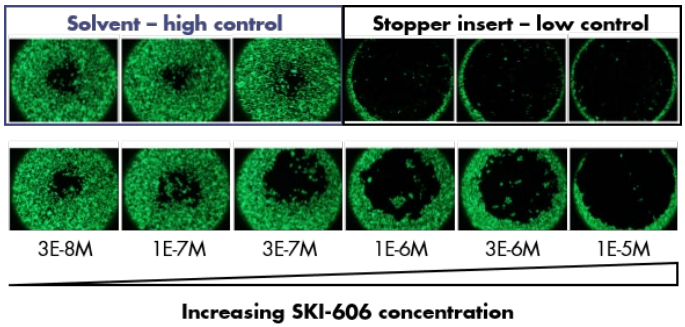
The Cell Migration Assay is used to investigate the initial phase of the metastasis process wherein tumor cells migrate away from the primary tumor site. The assay is comparable to the widely used scratch assay.

- High reproducibility
- High-throughput compatible
- Combination treatment possible
- Deliverable: IC_{50} values of test compounds on anti-migratory activity



ORIS Assay procedure

(A) Tumor cells are seeded onto collagen I-coated multi-well plates equipped with a barrier that prevents cell adherence to the centre of each well. After 18 hours, the barrier is removed (C) revealing a clear region in the center of the well. During a 24 to 48 hour incubation period in the presence of test compound (D), tumor cells migrate into this detection zone (E) which is monitored by fluorescent labeling of cells (F).



Example of inhibition of cell migration

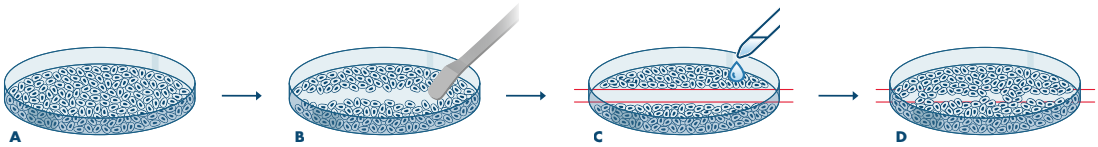
The c-Src inhibitor, SKI-606, was incubated with breast cancer cells for 48 hours and the migration of cells into the detection zone was visualized by fluorescence photography. Low control = removal of the cell barrier at the end of the test compound incubation.

Alternative readout: realtime monitoring of cell migration with IncuCyte

Cell Migration Assay (Wound Healing/Scratch Assay)

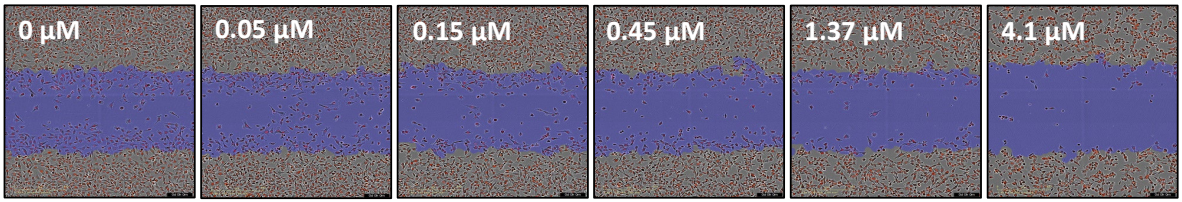
Measures the impact of compounds on undirected cell migration after making a wound/scratch on the cell monolayer. The assay is suitable for investigating tumor and normal cells.

- Real-time monitoring of cell migration
- Combination treatment is possible
- Deliverable: IC_{50} values of test compounds and images of wound closure



Assay procedure

(A) Tumor cells grow to monolayers. (B) Using the WoundMaker, homogeneous scratches are created mechanically with pins in a 96 well format. (C) After the application of the test compound, plates incubate in the IncuCyte S3 instrument for real-time quantification of tumor cells migrating into the scratch zone (D).



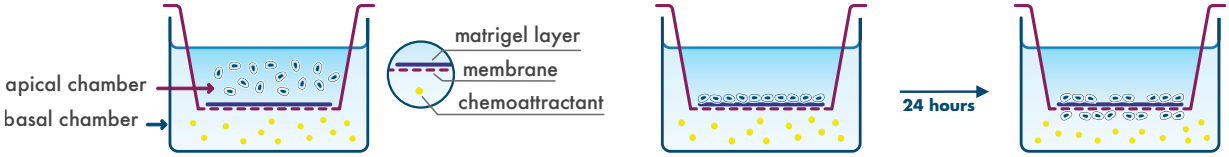
Effect of inhibition of cell migration on HT-1080 cells

Representative images of dose-dependent effect of the drug, Cytochalasin D, on wound healing at 8h time point. The blue areas represent the initial wound scratches.

Invasion Assay

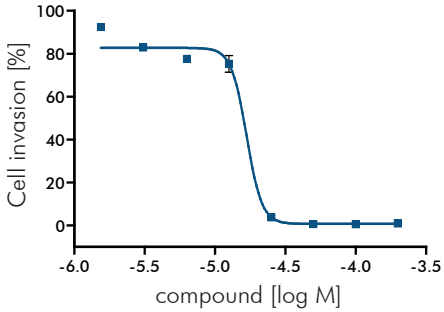
Metastasis is a multi-step process in which tumor cells enter the vascular or lymphatic circulation to find a new location to form secondary tumors. The invasion process requires cells to migrate into neighboring tissue via degradation of the extracellular matrix proteins and transversing the basement membrane, which in this assay is mimicked by a membrane in a trans-well insert.

- High-throughput compatible
- Combination treatment is possible
- Deliverable: IC_{50} values test compounds on anti-invasive activity



Assay procedure

Cells are seeded into the apical chamber of a transwell system. The basal chamber is filled with cell culture media supplemented with the fetal calf serum that serves as a chemoattractant. The membrane separating the chambers is coated with extracellular matrix proteins. Within 24 hours incubation in the presence or absence of test compound, cells invade the basal chamber through the membrane and are quantified via Calcein staining.



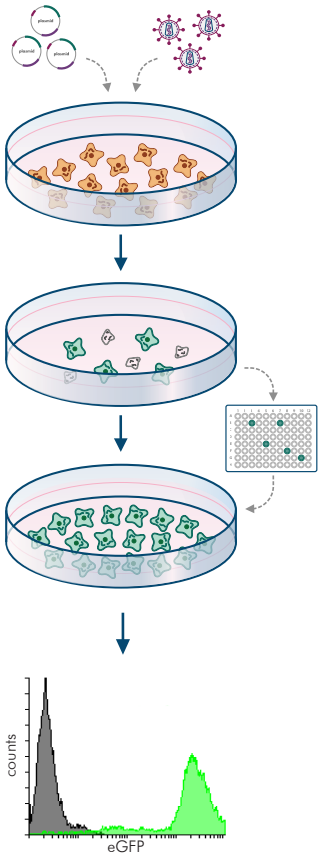
Example of Gefitinib treatment of cell line

Human breast cancer cells were incubated with Gefininib for 24 hours after which cells invading the basal chamber were quantified.

Cell Line Generation

Stably engineered cell lines fill many roles in research including the investigation of the effects of genes that are 'knocked-in' or 'knocked-out' and for labeling cells to enable their tracking in in vivo experiments.

- Standard transfection methods with lipofection/nucleofection or Ca_2PO_4
- Lentiviral transduction that allows for highly efficient target gene expression
- Single cell clones or population expression available
- Deliverable: 2 million cells and expression data



Gene information: custom made plasmids, eGFP, renilla or firefly luciferase

Gene delivery: lentiviral, lipofection, nucleofection

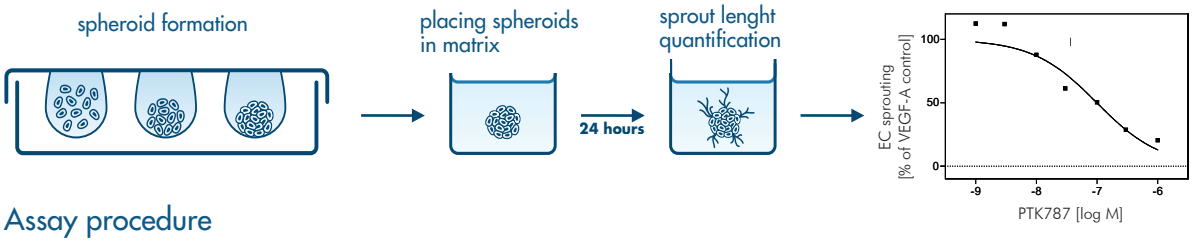
Selection: polyclonal or monoclonal

Expression analysis: Flow cytometry, Western blot, luciferase activity

Cellular Angiogenesis Assay

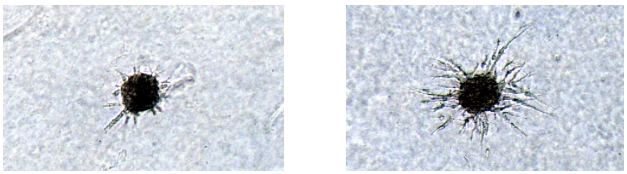
The spheroid-based Cellular Angiogenesis Assay is suitable for the investigation of early events of angiogenesis – the sprouting of new blood vessels from endothelial tissue.

- Induction by VEGF-A, FGF-2, HB-EGF, deferoxamine (chemical hypoxia) or others
- Performed with primary human umbilical vein endothelial cells (HUVEC).
- Deliverable: IC_{50} values of the anti-or pro-angiogenic capacity of compounds



Assay procedure

Endothelial cells aggregate to form spheroids in a hanging drop system. The spheroids are pipetted into 24 well plates in a collagen matrix to which the test compounds and stimulation factors are added. During a 24-hour period, vessels sprout from the spheroids and are quantified by measuring their length to determine the cumulative sprout length.



Vessel sprouting

Unstimulated endothelial cell spheroids show limited sprouting into the surrounding collagen matrix (left). However, sprouting is induced by pro-angiogenic factors like VEGF-A (right).

The number and length of the sprouts correspond to the angiogenic activity of the endothelial cells. Pro-angiogenic compounds induce sprouting; whereas angiogenesis inhibitors prevent new vessel formation.

Let's Discover Together.

Recombinant Proteins

- Kinase proteins
- Epigenetic proteins
- Substrates
- Custom-tailored protein production

Target-Specific Assays

- Biochemical and cell-based assays
- Enzymatic activity testing
- Protein: Protein Interaction assays
- Receptor Biology

Cellular Oncology

- 2D and 3D proliferation assays
- Drug combination screening
- Invasion and migration assays
- Angiogenesis assay

Biophysical Assays

- Surface Plasmon Resonance
- Thermal Shift Assay
- Isothermal Titration Calorimetry
- Microscale Thermophoresis

In Vivo Pharmacology

- In Vivo Hollow Fiber Model
- Xenograft models
- Orthotopic models
- Metastasis models

Safety & Adme-Tox

- Cardiac Safety Panel
- CYP inhibition
- PK/PD studies
- In Vitro Safety Panel

Integrated Drug Discovery

- Target research
- Hit identification
- Hit-to-Lead
- Lead optimization

Biomarker Discovery

- Genomic biomarkers
- Protein biomarkers
- Immunophenotyping

Immuno-Oncology

- In Vitro Killing Assays
- Syngeneic Mouse Models
- Propriatary Tumor Models
- Immunophenotyping

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