



# CELL-BASED ONCOLOGY ASSAYS

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

Let's discover together.



# Cell-based Oncology Assays

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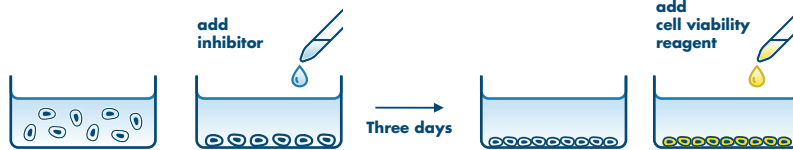
Reaction Biology offers a variety of phenotypic assays for the discovery of anti-cancer drugs based on human tumor cell lines. All of our assays are high-throughput compatible and can be customized according to your specific requirements.

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# 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.

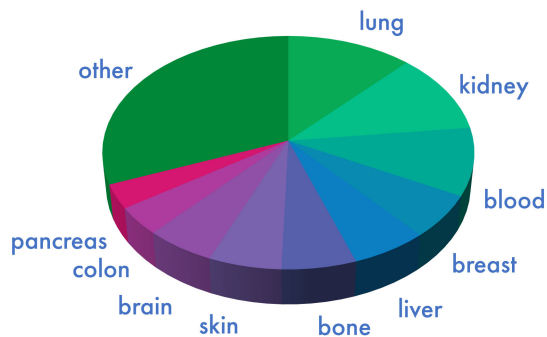
- Free choice option for the screening of any number of compounds on your choice of cell lines
- Readouts: CellTiter-Glo™ and other methods including ATPlite, CyQUANT, BrdU incorporation, MTT, Alamar Blue, and WST-8 readouts as well as real-time kinetics measured via IncuCyte
- Deliverable: IC50 values 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 CellTiter-Glo™ based on the amount of ATP present.

Collection of ~1000 cell lines, with over 600 of cancer origin



Over 1000 cell lines are available for drug testing in our cell proliferation and viability assays.

We also have Biosafety Level 2 rated facilities and can work with BSL-2 materials. Cell lines that are not in our panel can be established according to the specific research needs of our customers.

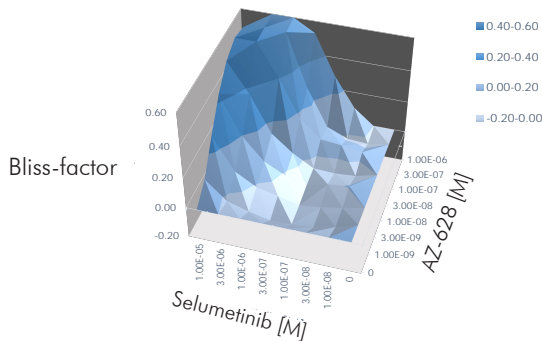
Please inquire for a list of our cell lines.



# Combination Therapy with Cell Proliferation Assay

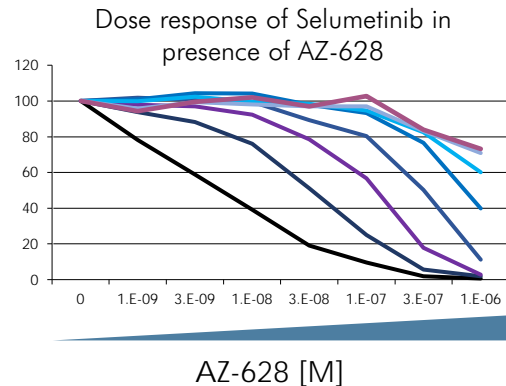
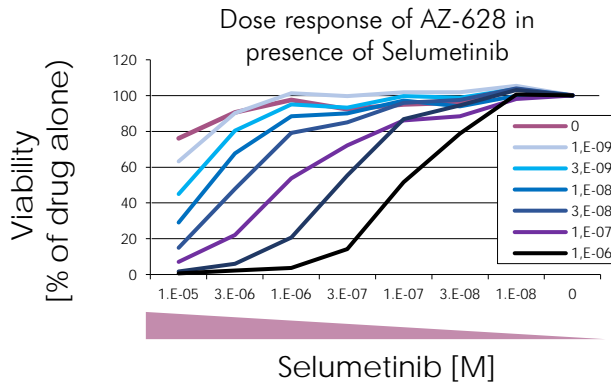
Combining drugs that affect different synergistically-acting targets have become a promising new strategy for cancer therapy.

- Combination drug testing can be performed with all of our cell-based oncology assays
- High-throughput option available
- Deliverable: IC<sub>50</sub> 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

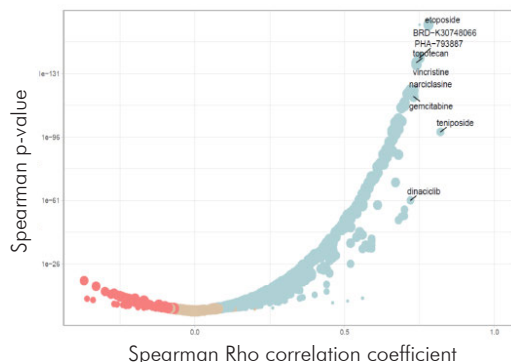
The addition of the second drug in increasing concentrations leads to reduced cell viability. In this example of mutual synergy, this effect can be seen for both drugs.

# Bioinformatics Analysis of ProLiFiler Data

Partnered with 4HF Biotech 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.

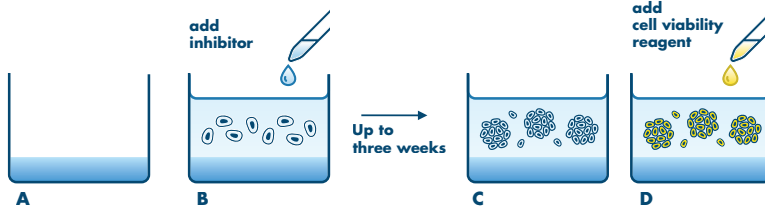
Gene symbol	Mutation counts in the Responder group	Mutation counts in the Non-Responder group	No Mutation counts in the Responder group	No Mutation counts in the Non-Responder group	Wilcoxon test p-value	Log2 of Wilcoxon test p-value	Mean absolute IC50 in non-mutated group	Mean absolute IC50 in mutated 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)

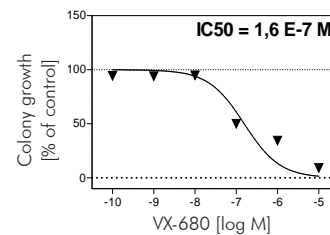
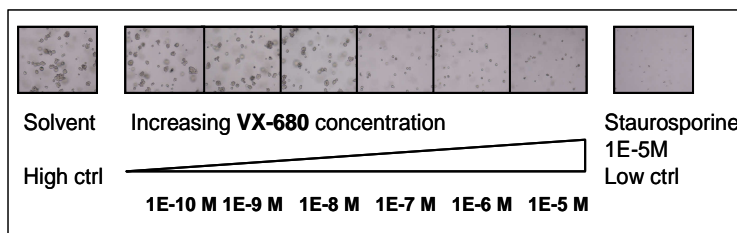
Anchorage-independent growth of cells is one of the hallmark characteristics of cellular transformation and uncontrolled cell growth. The soft agar allows the cells to grow to cell colonies independently of a solid surface.

- 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.



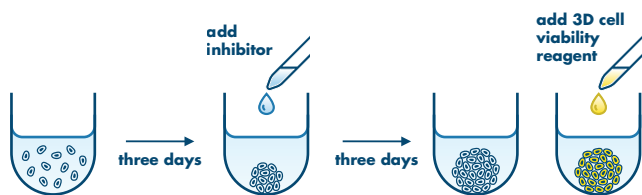
## Example of Aurora B inhibition in A549 cells

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

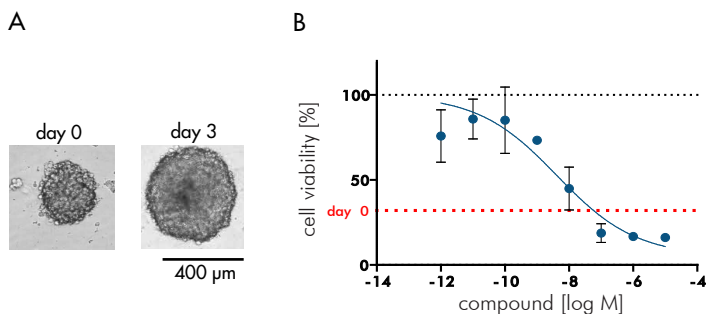


## Assay principle

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 CellTiterGlo-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 CellTiterGlo-3D™.



## Example: U87MG mono-spheroids

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

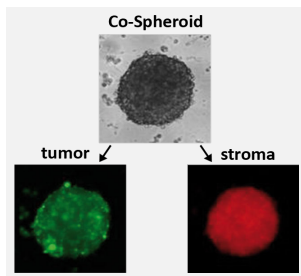
B. Dose-response of Trametinib treatment on U87MG mono-spheroids. Positive control (0% viability) is staurosporine treatment; negative control (100% viability) represents vehicle control. 'day 0' shows the number of viable cells present at the initiation of the experiment.

## 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		MiaPaCa-2
SK-NF-I		HT-29		LnCap	Prostate	
SK-N-MC		SW620		Duodenum	A2058	Skin
U87MG		Hutu 80				
MCF-7	Breast	HT-1080	Fibrosarcoma	A375	Stomach	
MDA-MB-231		A498	Kidney	MDA-MB-435		
T47D		A427	Lung	Hs746T		
ZR-95-1		A549		NCI-N87		
HeLa	Cervix	H1299	Lung	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

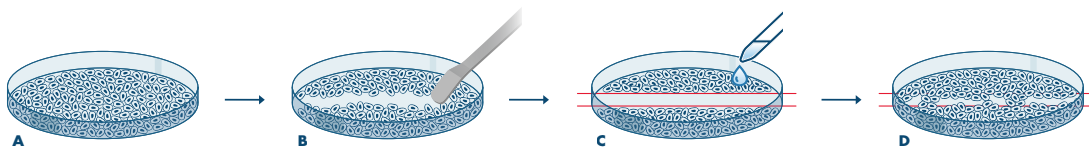
	HSS	HS27	NHDF
none	✓	✓	✓
A549	✓	✓	✓
DLD1	✓	✓	✓
HCT116	✓	✓	✓
LN229	✓	✓	✓
MCF7	✓	✓	✓
U87MG	✓	✓	✓

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

# Cell Migration Assay (Wound Healing/Scratch Assay)

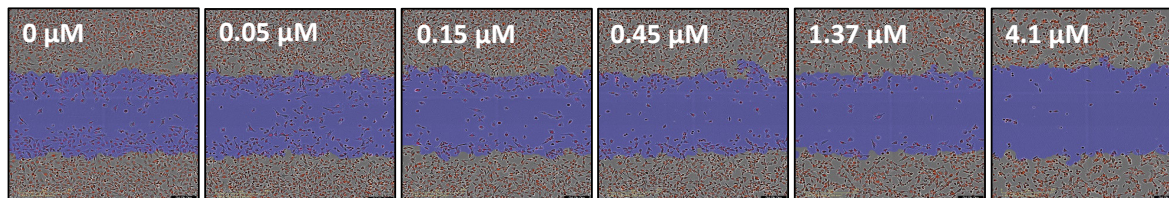
Measures the impact of compounds on undirected cell migration after making a wound/scratch on the cell monolayer. The assay is suitable for investigating tumor and normal cells.

- Real-time monitoring of cell migration
- Combination treatment is possible
- Deliverable: IC<sub>50</sub> 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).



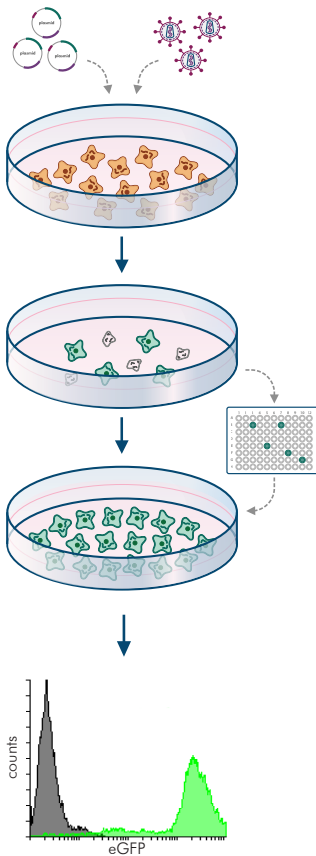
## Effect of inhibition of cell migration on HT-1080 cells

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.

# 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  $\text{Ca}_2\text{PO}_4$
- 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

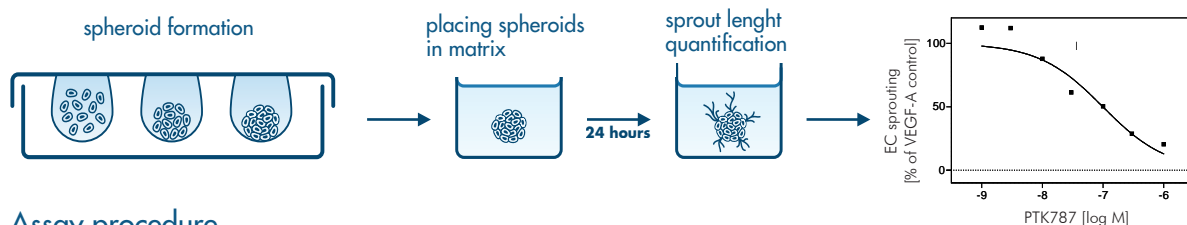
Selection: polyclonal or monoclonal

Expression analysis: Flow cytometry, Western blot, luciferase activity

# Cellular Angiogenesis Assay

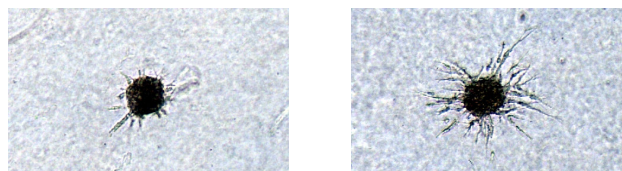
The spheroid-based Cellular Angiogenesis Assay is suitable for the investigation of early events of angiogenesis – the sprouting of new blood vessels from endothelial tissue.

- Induction by VEGF-A, FGF-2, HB-EGF, deferoxamine (chemical hypoxia) or others
- Performed with primary human umbilical vein endothelial cells (HUVEC).
- Deliverable: IC<sub>50</sub> 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.

# Notes

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# LET'S DISCOVER TOGETHER.

## Recombinant Proteins

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



## Biochemical Assays

- Kinases, Epigenetic Enzymes
- Protein: Protein Interaction assays
- Metabolic & Pathway
- Receptors & Channels



## Cell-Based Assays

- 2D and 3D proliferation assays
- Drug combination screening
- 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 & Toxicology

- In Vitro Safety Panel
- Cardiac Safety Panel
- Cytochrome P450
- Maximum-Tolerated Dose
- GLP Regulatory Toxicology



## Integrated Drug Discovery

- Antibody Drug Conjugates
- RAS Drug Discovery
- Targeted Protein Degradation



## Biomarker Discovery

- Genomic biomarkers
- Protein biomarkers
- Immunophenotyping



## Immuno-Oncology

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



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