

3D Tumor Spheroid Assay

➤ Spheroids as *in vitro* tumor surrogates

Three-dimensional tumor cell culture has been shown to mimic the physiological cancer situation more closely than growth on a flat surface. Spheroid analysis has evolved as one of the major 3D methods of choice for compound analysis due to multiple advantages:

- Cells autonomously assemble based on endogenous adhesion and matrix proteins, not requiring artificial matrix addition.
- Spheroidal structure challenges compounds to penetrate typical cell conglomerate barriers.
- Combination of different cell types for co-spheroid studies possible.
- Compatible with high-throughput analysis.

➤ The ProQinase “3D Tumor Spheroid Assay”

- Spheroid formation in low attachment u-bottom 96-well plates
- Viability assessment via luciferase-activity of Firefly or Renilla luciferase labeled cells.
- Available as mono- or co-spheroids.
- Simultaneous detection of two cell types in co-spheroids possible.
- Highly sensitive cell detection also allowing for low tumor/stroma ratios.

		Renilla Luciferase labelled stroma cells			
		none	HS5	HS27	NHDF
Firefly Luciferase labelled tumor cells	none		✓	✓	✓
	A549	✓	✓	✓	✓
	DLD1	✓	✓	✓	✓
	HCT116	✓	✓	✓	✓
	LN229	✓	✓	✓	✓
	MCF7	0	0	✓	✓
	U87MG	✓	✓	✓	✓

Table 1: Cells currently available for mono- or co-spheroid studies. (✓ = good aggregates; 0= loose association)

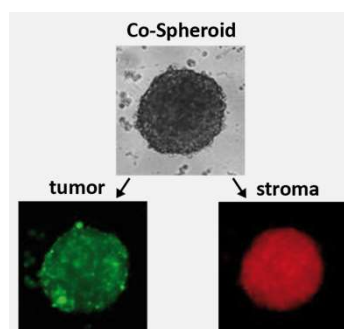
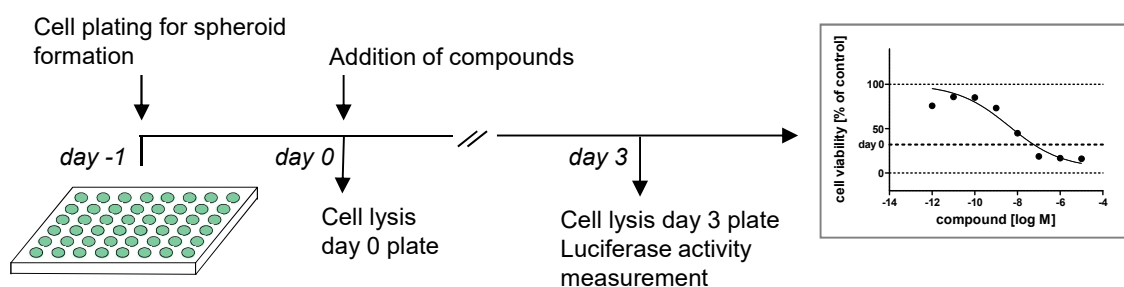


Figure 1: Co-Spheroid of 1000 U87MG tumor cells (Calcein stain) and 2000 HS27A stroma cells (RFP stain) one day after cell seeding.

➤ You ship your compounds – ProQinase performs the testing



- IC50 values are determined by testing 8 compound concentrations in deca- or semi-logarithmic steps (each concentration in duplicates).
- Quality assurance is provided by calculation of Z' factors for Low/High controls on each assay plate.