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.

<table>
<thead>
<tr>
<th>Renilla Luciferase labelled stroma cells</th>
<th>none</th>
<th>HS5</th>
<th>HS27</th>
<th>NHDF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Firefly Luciferase labelled tumor cells</td>
<td>none</td>
<td>A549</td>
<td>DLD1</td>
<td>HCT116</td>
</tr>
<tr>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
</tbody>
</table>

**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.

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