Soft Agar Growth Assay Service
(Anchorage-independent cell growth)

ProQinase TARGETING CANCER

Cellular Assay Service

➢ The Role of Anchorage-independent Cell Growth in Tumor Development

Anchorage-independent cell growth measured in the soft agar assay is the gold-standard for cellular testing of potential therapeutic agents in oncology, since Hamburger and Salmon developed the human tumor clonogenic assay in 1977[1]. Normal epithelial cells are supported by basement membranes providing survival and proliferative signals and undergo apoptosis when placed in suspension culture. Cancer cells, in contrast, evade attachment-regulated apoptosis, leading to uncontrolled proliferation. In order to discover agents that revert cell transformation and inhibit anchorage-independent cell growth, the ProQinase soft agar assay in the presented format combines sophisticated 3-dimensional cell culture with high throughput and reliable quantification.

[1] Hamburger and Salmon, Science (1977) 197:461

➢ Assay Procedure

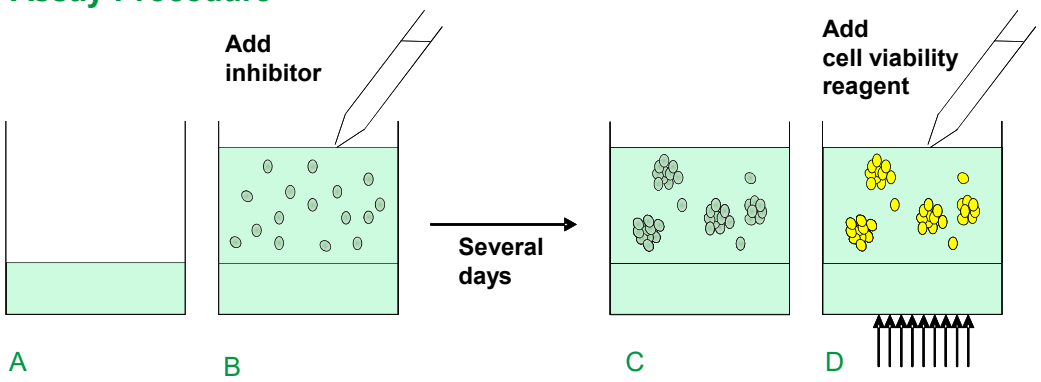

Figure 1: Assay procedure.
Wells of a 96-well plate are coated with 0,6 % soft agar (A), followed by seeding of the cells in 0,4 % soft agar (B). After the agar has solidified, compounds are added (B) and cells are incubated several days until colonies have formed in the solvent control (C). Subsequently, a cell viability reagent is added and fluorescence is measured as an indirect quantification of colony growth in soft agar (D).

➢ Study Example

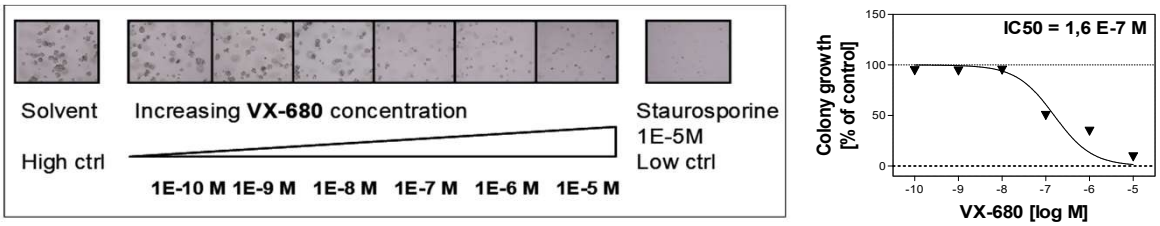

Figure 2: Study example.
The Aurora B inhibitor VX-680 was tested for inhibition of the soft agar growth of A549 non small cell lung cancer cells at indicated concentrations. Cells were left to form colonies for 5 days, photographed and stained with a fluorescent cell viability agent. Fluorescence was quantified and, for analysis of IC50 values, was expressed as percentage of colony growth in the presence of solvent alone.

The Soft Agar Growth Assay Service is currently established for 101 cell lines (see List of Cell Lines for Soft Agar Assays). Further cell lines and conditions can be established upon request. The assay is available to determine IC_{50} values (8 concentrations in duplicates).