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

REACTION BIOLOGY

CELL-BASED ASSAY SERVICES

## ➢ 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 Reaction Biology 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

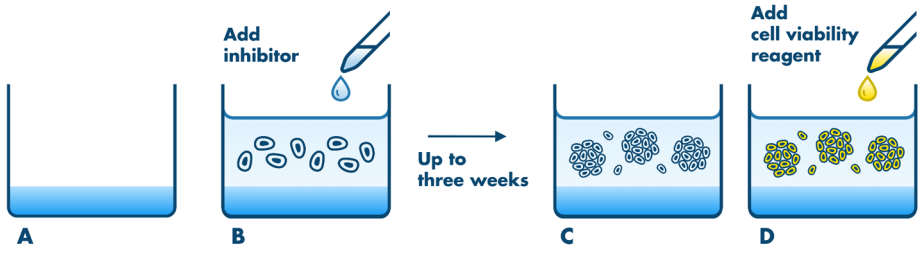


***Figure 1: Assay procedure.***
*Wells of a 96-well plate are coated with 0,6 % soft agar (A), followed by seeding of the cells in 0,4 % soft agar (B). After the agar has solidified, compounds are added (B) and cells are incubated several days until colonies have formed in the solvent control (C). Subsequently,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

## ➢ Study Example

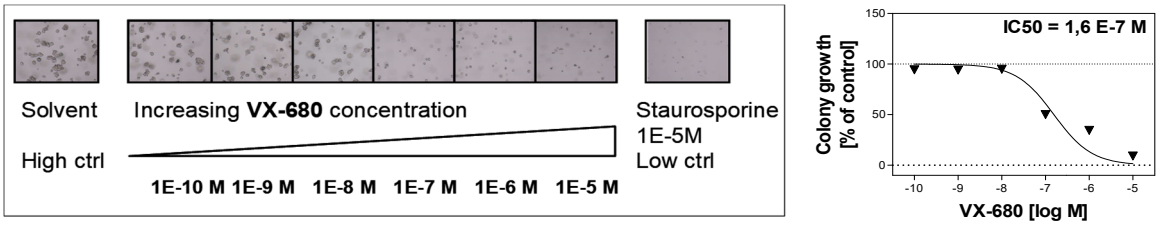


***Figure 2: Study example.***
*The Aurora B inhibitor VX-680 was tested for inhibition of the soft agar growth of A549 non small cell lung cancer cells at indicated concentrations. Cells were left to form colonies for 5 days, photographed and stained with a fluorescent cell viability agent. Fluorescence 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. Cell lines and conditions can be established upon request. The assay is available to determine $IC_{50}$ values (8 concentrations in duplicates).