

# Tumor Cell Proliferation Assay

## Materials and Methods

### Materials:

SK-BR-3 breast adenocarcinoma, human (ATCC # HTB-30)  
Doxorubicin (Sigma # D1515)  
AlamarBlue (Invitrogen # DAL1025)

### Methods:

#### Step 1:

SK-BR-3 cells are seeded at 4500 cells/ 100 $\mu$ l /well on 96-well tissue culture treated assay plate. Cells are incubated at 37°C and 7% CO<sub>2</sub> overnight to allow them to recover and reattach.

#### Step 2:

Next day make series of dilution of tested compound in 0.1% DMSO in growth medium and treat SK-BR-3 cells with tested compound for 72 hours. Each compound is done in a minimum of triplicate at 8 concentrations. Antitumour antibiotic Doxorubicin is used as a control proliferation inhibitor.

#### Step 3:

After treatment, SK-BR-3 cell proliferation is measured by fluorescent quantitation of AlamarBlue reagent. Our experiment has shown that the fluorescence intensity of AlamarBlue reagent is directly proportional to cell number. To perform the AlamarBlue assay, 10  $\mu$ l of AlamarBlue reagent is added to each well and the plate is incubated at 37°C for an additional 1 hour.

#### Step 4:

Fluorescence intensity is measured at an excitation of 530 nm and an emission of 590 nm using a BioTek Synergy<sup>TM</sup> 2 microplate reader. The fluorescent intensity data are analyzed using the computer software, Graphpad Prism. Quantitative IC<sub>50</sub> will be determined.

## Assay results

Effect of Doxorubicin on human breast cancer cell SK-BR-3 proliferation

