FLUORESCEIN DIACETATE ASSAY

Fluorescein Diacetate, which is dissolved in acetone, is converted by unspecific esterases of vital cells to fluorescein, which has a bright green fluorescence. The polar fluorescein is trapped in cells with intact surface membranes. This fluorescence can then be measured by standard flow cytometry.

Methods:

1x10^6 cells were suspended with drug in a final volume of 1ml of RPMI supplemented with 10% FBS, and incubated for 2 hours at 37 C. AraC was used at concentrations of 100 and 300um. Daunorubicin was used at concentrations of 3 and 10um. Controls consisted of cell suspensions incubated without drug.
Half of the suspensions were incubated with 2.5um PSC in addition to drug.
Following the 2-hour drug incubation, cells were washed, and resuspended in 1ml media. PSC was added to appropriate tubes, and all were allowed to incubate for a further 24 hours at 37 degrees.
Following incubation, cells were washed, resuspended in 0.5mls media. FDA was added to all tubes and allowed to sit at room temperature for 10 minutes, before being run on the FACSCAN.

Ref: Joachim W. Ellwart et al Cancer Research pp. 5722-5725 Oct 15th 1988