

# CELL PROLIFERATION VIABILITY ASSAYS

## HFF Cells Daudi Cells

### Methodology

The counting method of assaying for cell proliferation (viability) was carried out in roughly the same manner for all cell lines tested; that employed for HFF cells utilized in the herpes work is provided below as a representative example.

Twenty-four hours prior to assay, HFF cells were seeded in 6-well plates at a concentration of  $2.5 \times 10^4$  cells per well in minimum essential medium (MEM) containing 10% fetal bovine serum (FBS). On the day of the assay, drugs were diluted serially in MEM containing 10% FBS at increments of 1:5 covering a range from 100  $\mu\text{g/mL}$  to 0.03  $\mu\text{g/mL}$ . For drugs that were solubilized in DMSO, control wells received MEM containing 10% DMSO. The medium from the wells was then aspirated and 2 mL of each drug concentration was then added to each well. The cells were then incubated in a  $\text{CO}_2$  incubator at  $37^\circ\text{C}$  for 72 h. At the end of this time, the medium+drug solution was removed and the cells washed. One milliliter of 0.25% trypsin was added to each well and incubated until the cells started to come off of the plate. The cell-medium mixture was then pipetted up and down vigorously to break up the cell suspension and 0.2 mL of the mixture was added to 9.8 mL of Isoton III and counted using a Coulter Counter. Each sample was counted three times with two replicate wells per sample.

### Results

All Virutases except for CA with Daudi cells did not inhibit cell proliferation at drug levels at least as high as 50  $\mu\text{g/mL}$ , as shown below in Table II.

Table II. Cell Proliferation Inhibition Concentrations at 50% ( $\text{CP}_{50}$ ) of Virutase Drugs with Indicated Cell Lines

Virutase	$\text{CP}_{50}$ , $\mu\text{g/mL}$	
	HFF <sup>a</sup>	Daudi <sup>b</sup>
CA	71.2	<0.08
CGA	96	>50
HA	88.4	>50
HGA	>100	>50

<sup>a</sup>Human foreskin fibroblast cells. <sup>b</sup>Burkitt's lymphoma derived cells.