



## Homogeneous Cytotoxicity Assay

### ToxCOUNT™ (Validated Assay)

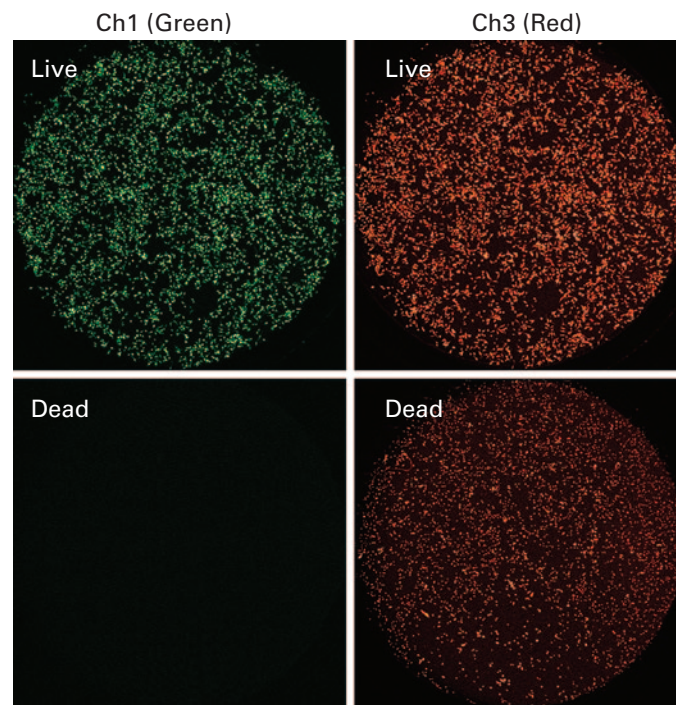
Assays that determine the health of cells are important in drug screening environments. The ToxCOUNT™ Cell Viability Assay Kit (Cat. # 18010) from Active Motif (Carlsbad, CA) is a simple, two-color assay to determine viability of cells in a population. The kit identifies live cells on the basis of intracellular esterase activity and plasma membrane integrity. Dead cells are identified through loss of plasma membrane integrity. The assay is suitable and validated for most eukaryotic cell types. In live cells the activity of intracellular esterases converts the nonfluorescent cell-permeant calcein AM to fluorescent calcein with an emission maximum at 515 nm. In dead cells, the ethidium homodimer-1 (EthD-1) enters cells with damaged membranes and fluorescence is enhanced by binding to nucleic acids, producing a bright red nuclear fluorescence with an emission maximum at 635 nm. EthD-1 is excluded by intact plasma membranes found with live cells. The **ImageXpress Velos** System provides an ideal platform for a fast, simple, and high-throughput homogeneous (no wash) cytotoxicity assay. Here we demonstrate an integrated image acquisition and analysis module with the flexibility important for automatic ToxCOUNT™ assay analysis under high throughput conditions.

## Assay Procedure

HeLa cells were plated in 96-well, black-walled, clear-bottomed polystyrene plates at a density of 2,500 cells/well in 50 µl of growth medium and incubated overnight at 37°C in a 5% CO<sub>2</sub> incubator. The growth medium was removed and the cells were exposed to various concentrations of saponin in PBS or MEM without serum (50 µl/well) for a 30 min treatment at 37°C in a 5% CO<sub>2</sub> incubator. After treatment the cells were stained with calcein AM and EthD-1 (50 µl/well, 0.5 µM for both) at 37°C in a 5% CO<sub>2</sub> incubator for 30 minutes. Each 96-well plate was scanned in 4 minutes at 5 x 5 micron sampling.

## Results & Discussion

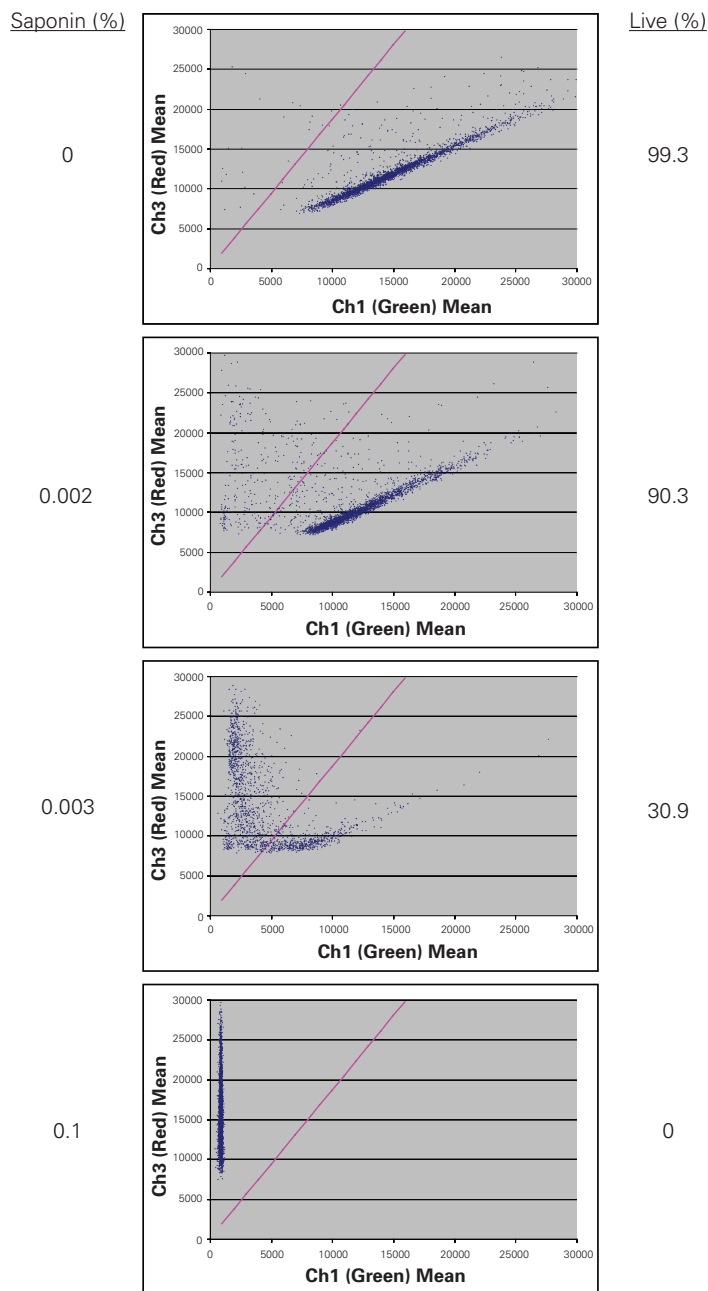
The **ImageXpress® Velos** System was configured with a 488 nm laser. The calcein (green) fluorescence emission was filtered through a 510-540 nm band pass filter for channel 1 (Ch1) and the EthD-1 (red) filtered through a 600 nm long pass filter for channel 3 (Ch3). A 96-well plate was placed into the plate nest of the **ImageXpress**



**Figure 1.** Whole well images. Top images are live cells in Ch1 (left) and Ch3 (right); bottom images are dead cells in Ch1 and Ch3. No signal is observed in Ch1 for dead cells.

**Velos** System for scanning and all data acquisition and process settings including PMT gains, cell enumeration conditions, and live/dead cell criteria were automatically configured by the ToxCOUNT™ method. Image analysis occurs concurrently with scanning and results can be viewed immediately afterward. The results are saved as a list file (.csv) enumerating cell-by-cell data for each well and a summary text file showing the percent live, percent dead, number of live cells, and number of dead cells in a plate layout format.

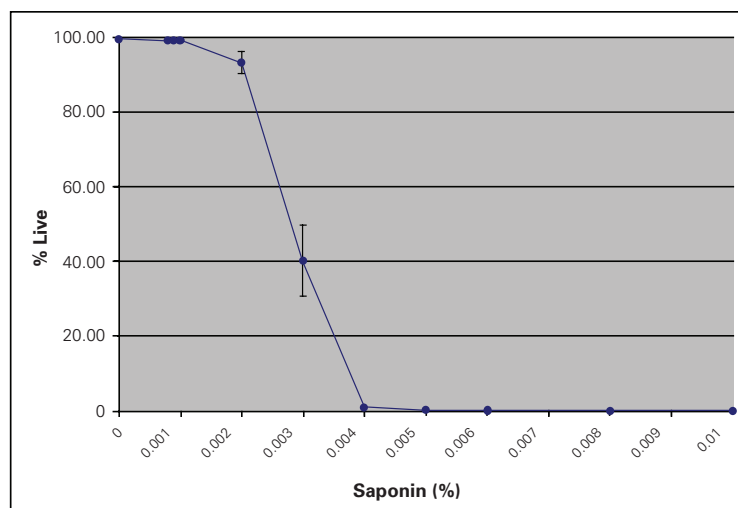
Whole well image results for live and dead cell control groups are shown in **Figure 1**. Cell by cell scatter plots are shown in **Figure 2**, with excellent discrimination between the two groups observed. Cells with a Ch1/Ch3 ratio >0.53 are grouped as live while cells with Ch1/Ch3 <0.53 are grouped as dead. The ratio is shown graphically as a magenta line in the **Figure 2** plots. Quantitation of the number of live and dead cells for the cytotoxicity assay is done by comparing the ratio of calcein signal (Ch1) to EthD-1 signal (Ch3). A heat map, in plate format is shown of the live percentage of cells after saponin treatment in **Figure 3**. The concentration dependent effect of saponin is shown in **Figure 4**.



**Figure 2.** Channel 1 (Green) Mean Intensity vs Channel 3 (Red) Mean Intensity. The data shown is from four separate wells treated with the indicated concentrations of saponin.

	1	2	3	4	5	6	7	8	9	10	11	12
A	98.9%	98.7%	98.7%	99.1%	87.6%	34.4%	0.4%	0.1%	0.0%	0.0%	0.0%	0.0%
B	99.3%	98.8%	99.4%	98.7%	90.3%	30.9%	0.2%	0.1%	0.0%	0.0%	0.0%	0.0%
C	99.5%	98.8%	99.1%	98.7%	95.5%	33.9%	0.8%	0.2%	0.1%	0.0%	0.0%	0.0%
D	99.5%	99.4%	99.1%	99.1%	94.7%	42.7%	1.0%	0.4%	0.2%	0.0%	0.0%	0.0%
E	99.8%	99.2%	99.3%	99.3%	97.0%	55.6%	1.7%	0.1%	0.0%	0.0%	0.0%	0.0%
F	99.4%	99.2%	99.1%	99.3%	93.2%	52.1%	1.3%	0.1%	0.0%	0.0%	0.0%	0.0%
G	99.8%	99.1%	99.0%	99.2%	92.4%	39.9%	0.8%	0.1%	0.0%	0.0%	0.0%	0.0%
H	99.2%	98.9%	99.1%	99.0%	93.6%	30.6%	1.1%	0.0%	0.0%	0.0%	0.0%	0.0%
	0	0.0008	0.0009	0.001	0.002	0.003	0.004	0.005	0.006	0.008	0.01	0.1

**Figure 3.** Heat map showing the percentage of live cells after saponin treatment. Column of eight wells was treated with the indicated concentrations of saponin (%).



**Figure 4.** Cytotoxicity of saponin. The results shown represent the mean  $\pm 1 \sigma$  of the live percentage of cells from columns 1-11 of Figure 3.

## Conclusions

The **ImageXpress Velos** System scanning platform and BlueImage cell analysis software has been demonstrated for cytotoxicity studies using the ToxCount™ Cell Viability assay kit. The results show that for HeLa cells the dye concentrations for this assay can be reduced to a final concentration of 0.5  $\mu$ M for both calcein AM and EthD-1. The ability to do whole-well scanning in a homogeneous format at high throughput, enables the running of a cell-by-cell cytotoxicity assay as a primary screen. The **ImageXpress Velos** System is an attractive solution to those laboratories in need of more user friendly quantitation of cytotoxicity screening assays. As shown here for the ToxCount™ assay, the **ImageXpress Velos** System provides a platform with integrated image acquisition and analysis modules with the flexibility important for developing, running, and validating image analysis procedures under high throughput conditions. The unique optics and scanning engine of this platform enables simple “plug and play” applications to meet the needs of life science research in both academia and industry.