

A New Tool for Routine Cell Counting:

Validation of Cell Count and Viability Assay

One of the simplest yet time-consuming tasks in many laboratories is the cell counting as a fundamental step for cell-based assays. Until now, cell counting and viability assay for many types of cell samples have been performed manually using hemocytometer.

Most of laboratories use trypan blue dye to distinguish viable cells from nonviable cells; only non-viable cells absorb the dye and appear blue or asymmetrical. The use of this stain, however, has the drawback of time-sensitivity. Viable cells absorb trypan blue over time, and can affect counting and viability results. The effort spent in performing these tasks can be a major cost in terms of time and resource utilization in laboratories. To overcome this labor cost situation, we have developed a plastic-chip based automatic cell counting device fully capable of performing cell counting as well as cell viability test associated with cell based assay.

Cell lines and culture conditions

CHO cell, 293 cell, Hela cell, SK-OV cell and NCI-H23 cell lines were used. Cells were grown in defined medium in humidified 5% CO₂ incubator at 37 C. Cells were trypsinized using standard methods to prepare cell suspensions for counting.

Manual cell counting and viability

Manual counts were done using a Neubauer-type counting chamber. Cell samples were stained with trypan blue by mixing 50 µl of 0.4% trypan blue solution and 50 µl of cell suspension. Cell samples ranging from 10⁵/ml to 7 × 10⁶/ml were scored under light microscope. Total cell concentration was calculated using appropriate dilution factors and hemacytomer calculation factors.

FACS analysis

Cell viability was simultaneously analyzed in the C-Reader cell analysis system and FACScalibur (BD Biosciences) equipped with a 532 nm excitation and emission in FL2, according to standard procedure.

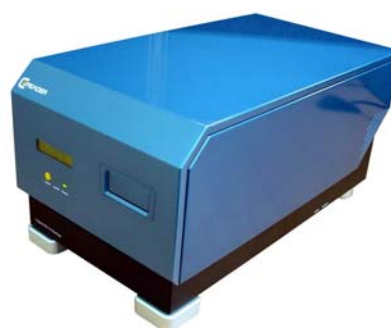


Figure 1. C-Reader: cell analysis system

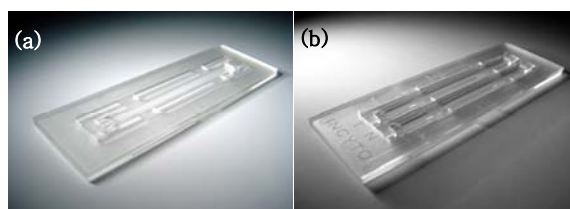


Figure 2. Disposable plastic chip for accurate cell number counting

(a) One channel chip for total cell number counting.

(b) Two channel chip for easy evaluation of cell viability.

C-Reader assay

Total cell counting

Five cell samples were stained using the following procedure. From culture samples ranging from 5 × 10³ to 1 × 10⁷ cells/ml, small amount of cell suspension more than 20 µl was mixed with the same amount of total cell stain solution (CRS-T01) and then 20 µl of the mixed sample was loaded onto the disposable plastic one channel chip, as shown in Fig. 2. (a). Within 1 min, total cell number in the sample was measured by the CounterStar Software as described in



Figure 3. Fluorescence image of CHO cells before image processing

the user's guide. Fig. 3 shows the fluorescence image of stained CHO cells as an example and the CounterStar counts the total cells from those images automatically.

Cell viability

Two channel chip was used for easy evaluation of cell viability (Fig. 2, b). For non-viable cell counting and total cell counting, non-viable cell stain solution (CRS-N01) and total cell stain solution (CRS-T01) were used with the same procedures as described above. Sixteen µl mixture of each stain solution (CRS-T01 and CRS-N01) and sample was loaded onto the disposable plastic two channel chip. Within 45 sec., viability of the cells was calculated automatically by the CounterStar Software.

Cell viability calculation

C-Reader cell analysis system can determine the viability of the cells automatically. First, the total cell number and second, non-viable cell number were measured and then the cell viability is calculated as follows.

$$\% \text{ Viability} = \frac{C_t \cdot M_t - C_{nv} \cdot M_{nv}}{C_t \cdot M_t} \cdot 100$$

% Viability : The percentage of viable cells in the original cell suspension.

C_t : The total concentration of cells in the C-Reader chip.

C_{nv} : The concentration of non-viable cells in the C-Reader chip.

M_t : The multiplication factor used for the total cell count.

M_{nv} : The multiplication factor used for the non-viable cell count.

Results and Discussion

The C-Reader cell analysis system shows good correlation to hemocytometer with correlation coefficient (R^2) of 0.99 in Fig. 4 and 0.996 in Fig. 5.

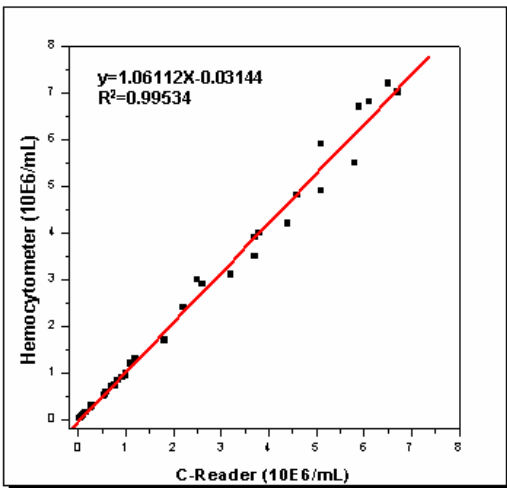


Figure 4. Correlation of total cell counting between hemocytometer and C-Reader cell analysis system using CHO cells

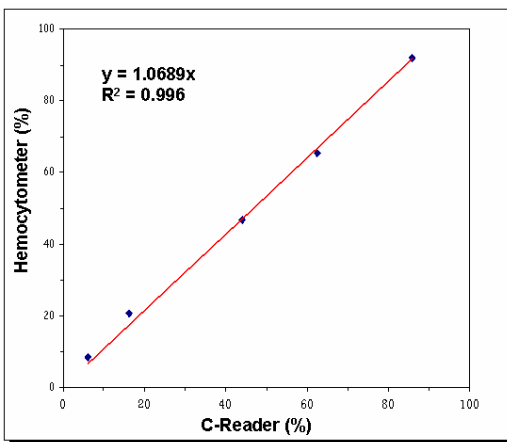


Figure 5. Comparison of viability between trypan blue/manual cont and C-Reader cell analysis system using 293 cells



It represents that the manual hemocytometer can be replaced by the C-Reader cell analysis system for the tedious counting job.

For the viability test, apoptosis-induced 3 cancer cell lines were analyzed with the FACS and the C-Reader. The results are shown in Fig. 6 and Fig. 7. H₂O₂-treated cells have a higher percentage of dead cells compared to the control cells.

Coefficient variation (CV) value ranged from 0.6% to 5.4% for the FACS and 0.3% to 1.5% for

the C-Reader cell analysis system. Comparison of the mean CV between the FACS analysis system and the C-Reader cell analysis system proved that the C-Reader is fully capable of performing mammalian cell viability test.

To verify the apoptosis model of this comparison, the terminal deoxynucleotidyl transferase-mediated FITC-dUTP nick end labeling (TUNEL) assay and the Annexin V-FITC, and DAPI staining assay could be performed.

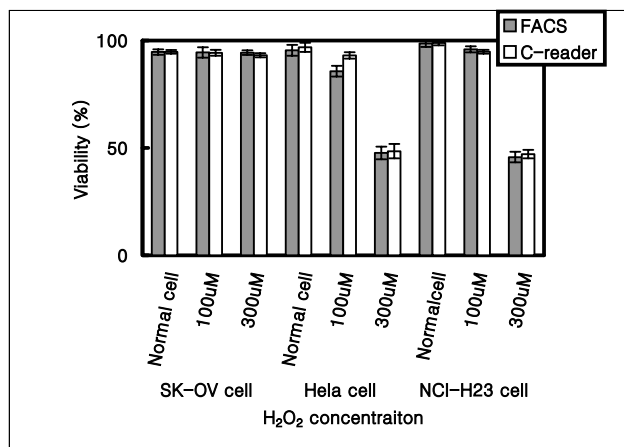


Figure 6. Comparison of the cell viabilities between C-Reader cell analysis system and FACS. SK-OV, HeLa and NCI-H23 cells were treated with 100, 300 µM H₂O₂ for 3 hours, then analyzed by C-Reader and FACS. Values are given as means ± SD of three experiments.

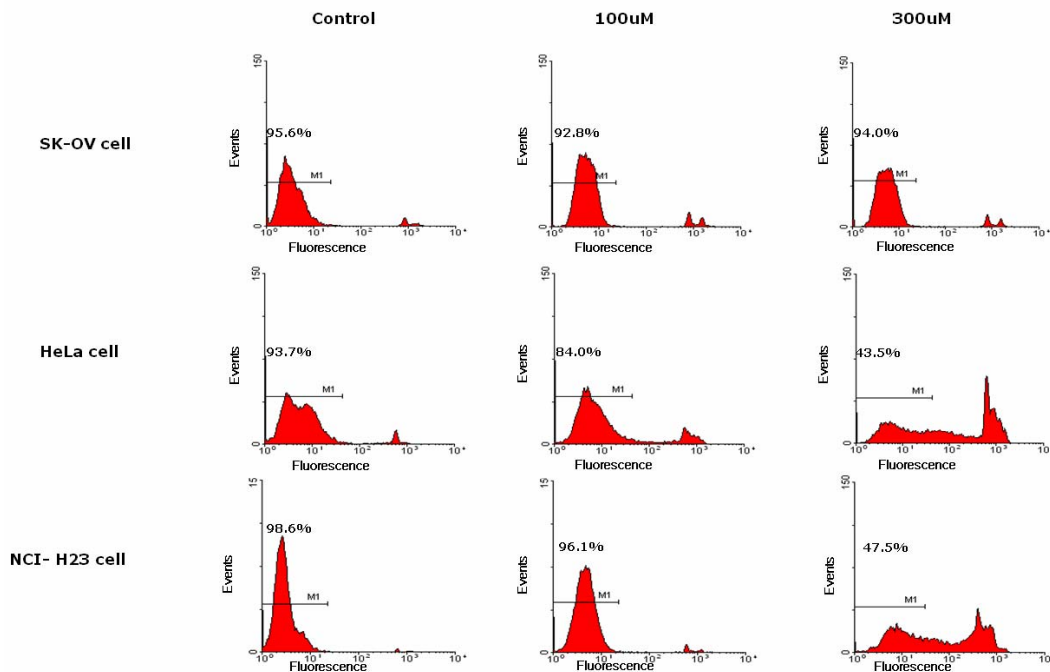


Figure 7. FACS analysis of H₂O₂-induced apoptosis in SK-OV, HeLa and NCI-H23 cells. Cells were treated with 100, 300 µM H₂O₂ for 3 hours or untreated as control. M1= viable cells. M2=non-viable cells.



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