The world's fastest automated cell counter

EVE™ *PLUS*
Application Note

Comparison of clumped cell counting using EVE™ PLUS vs. other automated cell counters.

General Description

EVE™ PLUS is the 2nd generation of EVE™, an image based automated cell counter. EVE™ PLUS is a benchtop automated cell counter that performs cell count and viability measurements using trypan blue solution.

Introduction

Cell counting is a fundamental step in most biology experiments. This may be necessary to standardize the cell concentration between samples in order to minimize variation and errors in downstream results.

In recent years, automated cell counters have become more popular because of disadvantages of manual count using hemocytometer. However, automated cell counters still have disadvantages that are hard to overcome.

Most image based automated cell counters capture images and then provide the results by imaging technology.

A typical problem is when these instruments is that it may give very inaccurate calculations when counting cells in a clumpy shape.

NanoEnTek, Inc. has developed EVE™ PLUS in order to overcome such a problem of the existing instruments.

EVE™ PLUS solves this problem by integrating advanced cameras with improved image analysis algorithms.

A comparison of clumped cell counting using EVE™ PLUS vs. other automated cell counter is presented in this application note.

Cell Line	Animal	Organ	Growth Properties	Detached Cell Morphology Type
HeLa	Human	Skin	Adherent	Single
NIH-3T3	Mouse	Embryo	Adherent	Single
U2-OS	Human	Bone	Adherent	Single
Jurkat	Human	Blood	Suspension	Single
KG-1a	Human	Blood	Suspension	Single
SH-SY5Y	Human	Brain	Adherent	Single
Hep G2	Human	Liver	Adherent	Aggregate
SCN2.2	Rat	Brain	Adherent	Aggregate
F9	Mouse	Embryo	Adherent	Aggregate
MCF7	Human	Breast	Adherent	Aggregate

Table 1. To verify the performance of the counting algorithm, ten cell lines in various forms and shapes were prepared.

Materials and Methods

Preparation of cell samples for counting

Eight adherent cell types and two suspension cell types were prepared, and their density was assessed using EVE™ PLUS. And four of the eight adherent cells were prepared in an aggregate type. <Table 1> contains more detailed information about tested cells.

Linearity test for nucleus stained cell count

It is inappropriate to compare the linearity of the count results with manual methods which have big errors and high variations, so it should be compared using the more accurate counting method, the nuclear staining cell count (ADAM-MC2). In order to confirm the linearity between the nuclear staining count and EVE™ PLUS, cells of high concentration were prepared, and serial dilution was performed. For this experiment, one single cell type (Jurkat cell) and one clumped cell type (SCN 2.2 cell) were used.

ADAM-MC2 instrument for nuclear staining cell count

ADAM-MC2, produced by NanoEnTek, is another automated cell counter that operates, based on staining mammalian cell DNA with fluorescence dye, Propidium Iodide (PI). PI is a popular red-fluorescent nuclear and chromosome counterstain. Since not permeant in to live cells, Propidium Iodide is also commonly used to detect dead cells in a population. To get a correct count result, ADAM-MC2 provides two kinds of staining solutions. AccuStain Solution T for the total cell counting is composed of fluorescent dye (PI) and lysis solution. AccuStain Solution N for the non-viable cell counting is composed of the fluorescent dye and PBS.

<Figure1> shows the principle of nucleus stained cell count with PI solutions.
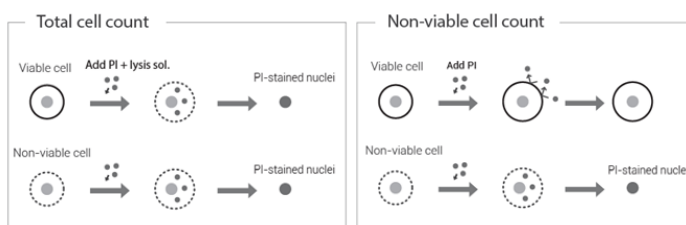

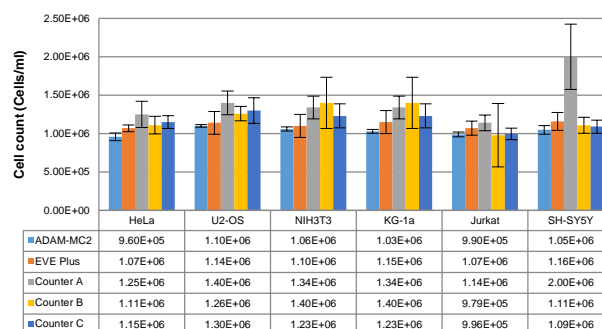**Figure 1. Principle of ADAM-MC2 staining solutions**
Left (AccuStain Solution T for total cell count) / Right (AccuStain Solution N for Non-viable cell count)

Results

Linearity test results of the nucleus staining counter

We compared the linearity test results of EVE™ PLUS to ADAM-MC2 (nucleus staining counter) using a sample serially diluted with two different shaped cells of high concentrations. For each cell type tested, measurements obtained correlated strongly with the linear cell density as indicated as the R^2 values ($R^2 \geq 0.99$; Figure 2). This results confirm that EVE™ PLUS provides accurate results not only in single cells but also in aggregated cells. **<Figure 2>** shows the linearity results for both cell types.

(A) Jurkat cell (single cell type)

$$y = 0.9724x$$
$$R^2 = 0.9994$$

(B) SCN 2.2 cell (aggregate cell type)

$$y = 0.9614x$$
$$R^2 = 0.9996$$

Figure 2. Results of linearity comparison between two different cell counter.
Data shown are average of five replicates of tests. Error bars represent mean and standard deviation.

Comparison of cell count results among other cell counters

We compared the cell count performance test of EVE™ PLUS to other market players (cell counters manufactured by other companies), and at the same time, the results by ADAM-MC2 were used as a standard. The concentration of the cells was processed to the extent that customers mainly use. Assessment of cell number and viability using other instruments was performed according to the manufacturer's recommended methods. Ten kinds of cells were classified into two groups according to cell types, and then the experiment was performed. The results are shown in **<Figure 3>**. This figure shows that EVE™ PLUS is a device that shows the nearest performance to the nucleus staining cell counting results and low CV in all cells.

(A) Single cell type

	HeLa	U2-OS	NIH3T3	KG-1a	Jurkat	SH-SY5Y
ADAM-MC2	9.60E+05	1.10E+06	1.06E+06	1.03E+06	9.90E+05	1.05E+06
EVE Plus	1.07E+06	1.14E+06	1.10E+06	1.15E+06	1.07E+06	1.16E+06
Counter A	1.25E+06	1.40E+06	1.34E+06	1.34E+06	1.14E+06	2.00E+06
Counter B	1.11E+06	1.26E+06	1.40E+06	1.40E+06	9.79E+05	1.11E+06
Counter C	1.15E+06	1.30E+06	1.23E+06	1.23E+06	9.96E+05	1.09E+06

(B) Aggregate cell type

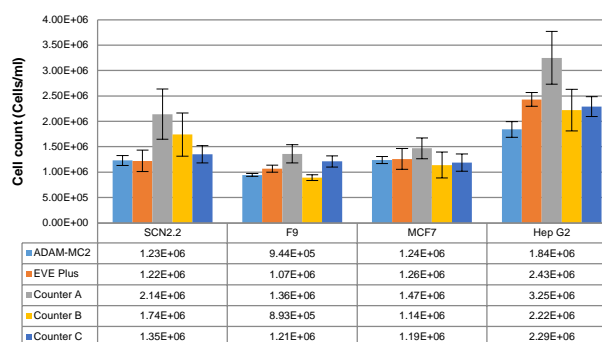|	SCN2.2	F9	MCF7	Hep G2
ADAM-MC2	1.23E+06	9.44E+05	1.24E+06	1.84E+06
EVE Plus	1.22E+06	1.07E+06	1.26E+06	2.43E+06
Counter A	2.14E+06	1.36E+06	1.47E+06	3.25E+06
Counter B	1.74E+06	8.93E+05	1.14E+06	2.22E+06
Counter C	1.35E+06	1.21E+06	1.19E+06	2.29E+06

Figure 3. Comparison of cell count result among other counters.
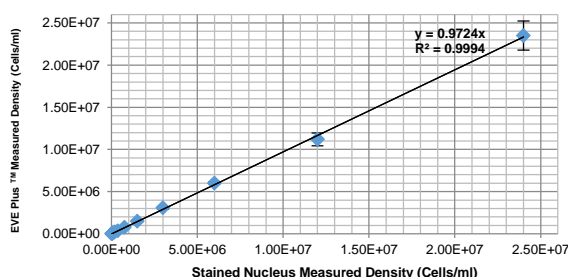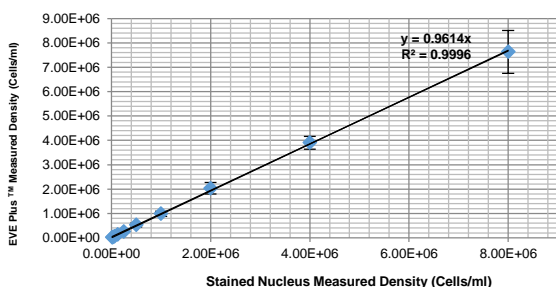Experiment was carried out by classifying into two groups according to cell types. Data shown are average of ten replicates of tests. Error bars represent mean and standard deviation.

Conclusions

EVE™ PLUS is the best performing basic cell counter for the following reasons.

- Most similar result to the result of cell counting the nucleus staining
- The best option for clumped cell counting
- Superior results compared to competitors
- Counts in a second (manual focus option)
- Repeatability of results guaranteed (auto focus option)
- Produces accurate results in most cells
- Low CV through high-end algorithm
- Relatively inexpensive disposables
- Auto save function up to 500 results

EVE^{TM} PLUS Cell Counter FAQs

Q. How quickly does EVE^{TM} PLUS count cells?
A. 1 second or 10 seconds.
There are two options for cell count of EVE^{TM} PLUS.
Calculated the Manual Focus option, results are displayed within 1 second.
On the other hand, calculated with the Auto Focus option, results will appear in about 10 seconds.

Q. Which option would you recommend between two focus options?
A. It is recommended that you use the Auto Focus option until you are familiar with the focus guide. If you are familiar with the focus guide, you may use Manual Focus option, which significantly can save time. This is because, samples of similar sizes are to be counted, the focus correction is virtually unnecessary.

Q. Cell size and cell counting range is ?
A. Cell concentration measurement ranges from 1×10^4 to 2×10^7 **cells/mL,** and cells with sizes ranging from **5 μm to 60 μm** may be counted with EVE^{TM} PLUS.

Q. Do I have to save data after every count like other cell counter brands?
A. No need to back up after every count. Up to 500 results are automatically stored in the DATA tab after counting. This function eliminates inconvenience of having to back up necessary data to a USB stick.

Q. Can I edit results?
A. Yes, the counted result can be edited in the DATA tab. Cell size gating can also be modified.

Q. What kind of samples can be counted with EVE^{TM} PLUS?
A. Basically, **rounded cells** that are between **5 μm to 60 μm** in size are countable. For example, **almost all kinds of mammalian cell line** can be counted, such as cancer cell lines, primary cells, red blood cells, PMBC, and some round type of yeast cells. However, it is not possible to count small bacteria and highly motile sperm.

Q. How does EVE^{TM} PLUS distinguish dead cell from living cells?
A. Since only dead cells are stained with **0.4% Trypan blue**, it is possible to discriminate through the contrast of the cells.

Q. How does EVE^{TM} PLUS count clumped cells?
A. EVE^{TM} PLUS searches for the surface of aggregated cells to recognize the curved portion as a boundary, and they are divided into the average size of the whole cells. This feature is enabled by high-resolution cameras and perfectly optimized algorithms.

Q. Why an activation code is necessary to use EVE^{TM} PLUS?
A. In recent years, EVE Slide replicas have been circulated in the market. Since the chip height of the replicas is not uniform due to poor quality, it makes it difficult to get correct counting results. Therefore, And to exclude the provision of false information through the procedure of authentication. To make sure you enjoy all the features of EVE^{TM} PLUS using authentic EVE slide products. EVE^{TM} PLUS will ask you to input an activation code.

Nous contacter

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NanoEnTek

OZYME
Des femmes et des hommes au service de vos recherches