Image Based Cell Viability Assay

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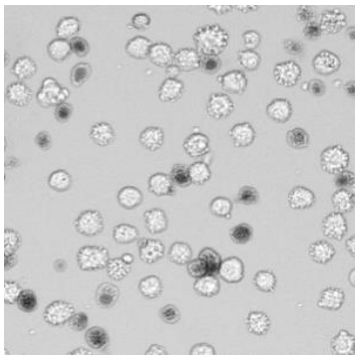


Application Note
AN-B004-XXI-13

Introduction

Cultivation of Cells and Cell based assays often require researchers to determine the amount of cells they are preparing for these assays. Furthermore, in routine subculturing, cell line development or upscale bioreactor cultivation, it is important to control the viability of the culture.
The Trypan Blue Viability Test is a widely used technique to determine the cell number and the culture viability. In a lot of cases this is performed by manually counting cells with a hemocytometer which is tedious and leads to subjective and biased results. Automated or semi-automated cell counters are available for this purpose and each one has individual advantages and disadvantages.
The Trypan Blue test is based upon the concept of dye exclusion: viable cells do not take up Trypan Blue, whereas it can pass the damaged membrane of dead cells. SYNENTEC has adopted the classical Trypan Blue staining method for its NYONE® and CELLAVISTA® cell imager: dead cells appear dark in the brightfield image, whereas live cells remain opaque. The Trypan Blue assay can be carried out as whole-well measurements in standard 96-well microplates with significantly increasing throughput, reducing the time-to-result, requiring smaller sample volumes and generating the least cost per sample compared to other existing assay formats.

Materials

Liquids

- Cell suspension
- PBS (w/o Ca^{2+} w/o Mg^{2+}) (e.g. Biochrom)
- Trypan Blue 0.02 % w/v in PBS (e.g. made of Gibco 0.4 % stock solution)

Equipment

- 96-well microplate with transparent bottom (e.g. Nunclon™ ; Thermo Scientific)
- Centrifuge with swing-out rotor (e.g. Eppendorf 5810)
- One of SYNENTEC's imaging systems (here: NYONE®)
- Micropipette and tips
- Microtube

There is no need to pre-incubate the liquids at 37 °C. Room temperature is sufficient.

You can use the remaining wells of a plate for new samples and measurements.

Procedure

1. Make sure that all liquids have room temperature at minimum. Pre-incubation at 37 °C is not mandatory but can also be used.

Try to reduce cell aggregates.

2. We recommend a final cell concentration (after adding Trypan Blue) of ca. $3 \cdot 10^5$ cells/mL. That is the most precise measurement range (fig. 2). If you expect a much higher cell concentration in your initial cell suspension, apply a pre-dilution with PBS^- in a microtube, according to the following dilution table:

Table 1: The following table shows the exemplarily volumes for 96-well full area and 96-well half are plates. The volumes are calculated to a final sample volume for at least 3 repetitions per cell suspension-sample (full area á 80 µL, half area á 40 µL). We recommend never taking less than 20 µL / cell suspension, otherwise the method error will increase significantly (data not shown).

				Full area 96-well plate (e.g. Nunclon™; Thermo Sci.)			Half area 96-well plate (e.g. Corning Costar # 3695)		
Expected cell density		Final dilution		PBS^- [µL]	Cells [µL]	TryB 0.02 % [µL]	PBS^- [µL]	Cells [µL]	TryB 0.02 % [µL]
$1 \cdot 10^7$	→	1:80	→	1560	40	1600	780	20	800
$5 \cdot 10^6$	→	1:40	→	760	40	800	380	20	400
$1 \cdot 10^6$	→	1:20	→	360	40	400	180	20	200
$5 \cdot 10^5$	→	1:10	→	160	40	200	80	20	100
$1 \cdot 10^5$	→	1:5	→	120	80	200	60	40	100
Less $1 \cdot 10^5$	→	1:2	→	0	120	120	0	60	60

3. Pipet the diluted and stained cell suspension into the wells of the plate (full area á 80 µL, half area á 40 µL). Three wells per samples are recommended to achieve significant and reliable statistics.

4. Incubate the mixture for 10 minutes at room temperature to ensure homogenous Trypan Blue staining.

5. Centrifuge the plate for one minute at 30 x g in the swing-out rotor.

6. Remove the plate carefully out of the centrifuge and place it into the sample holder of the NYONE® imager.

7. The measurement should be done within one hour after Trypan Blue addition. The cells remain stable during this period due to the low Trypan Blue concentration used in this assay.

Make sure that the acceleration-step and brake-step during centrifugation is as short as possible.

Avoid unnecessary agitation of the plate to prevent uneven cell distribution and cell movement inside the wells.

Avoid touching the plate bottom during handling steps. Keep the plate bottom as clean as possible (place it only on a clean spot) and prevent particle contamination to ensure optimal image quality.

Accuracy

To demonstrate the accuracy of the NYONE® and its YT-software®, we conducted experiments compared against the current gold standard of Cell Counters. We investigated the comparability with the results of our competitor. For this purpose, 12 samples with different cell densities were measured - in each case with the NYONE® and also with the competitor to determine the cell density. For the NYONE® a transparent 96-well full area plate was used and for the comparative system the samples were loaded in the cuvettes of the auto-sampler carrousel.

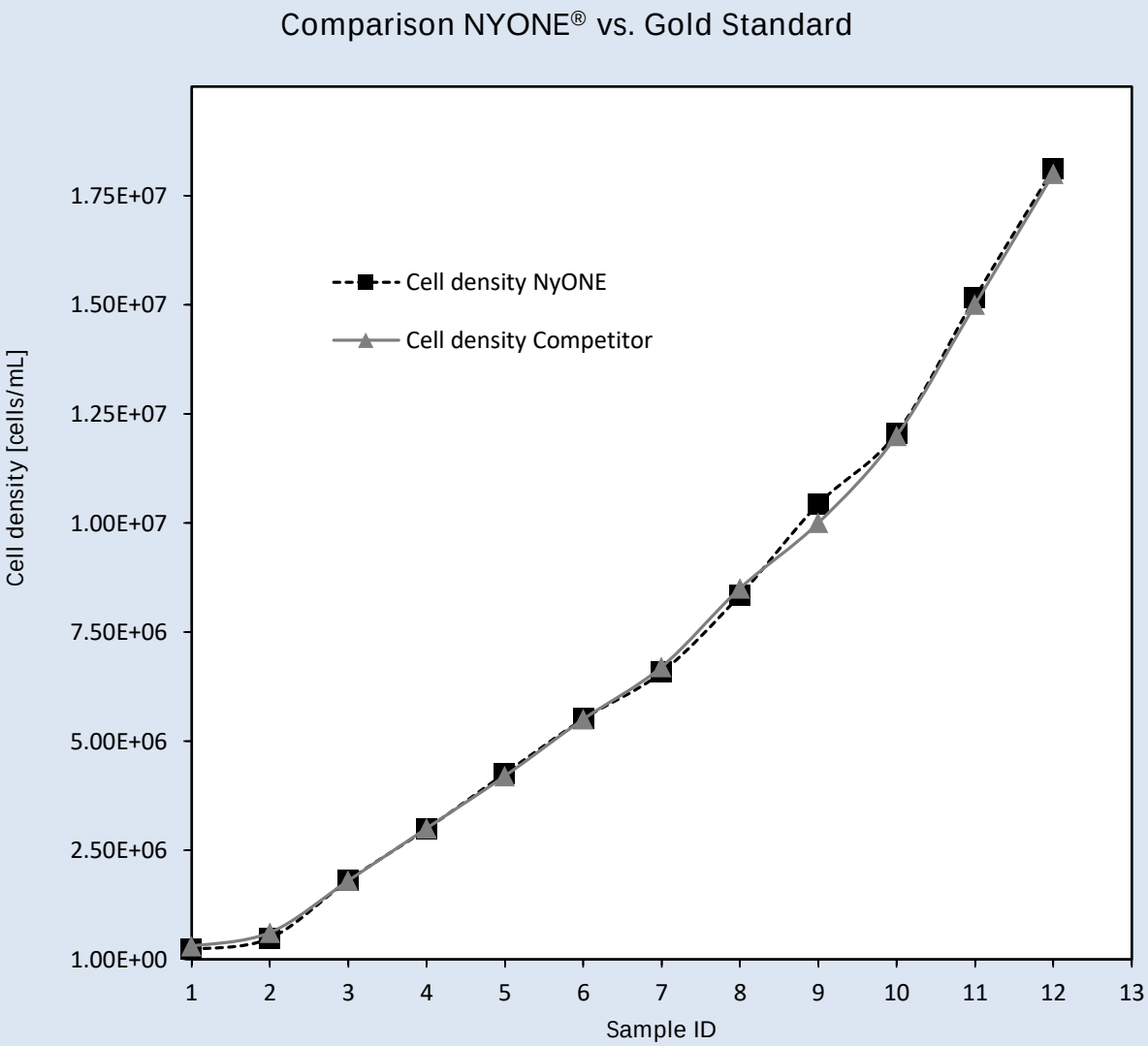


Fig. 1: Comparison of cell density determination between the NYONE® and the competitor.
Twelve samples (sample ID 1-12) of transfected CHO cells were counted with the NYONE® (green line) and with the gold standard in cell counting (gray line). Cell densities between 3.00E+05 and 1.80E+07 cells/mL were used.

The figure above (fig .1) shows the fairly exact matching of NYONE®'s achieved results compared to the competitor. The wide range of 30 thousand cells up to nearly 20 million cells per milliliter

was covered in this experiment. Here it can be seen that the accuracy of our measurement method is cell density-independent.

Reproducibility

In addition to the comparative studies, the reproducibility of the results was analyzed within one method. When measuring with the NYONE® eight replicates per sample-ID were taken and the standard deviation within those replicas was examined. This experiment also served to determine the valid cell density range for measurements with the NYONE® Imaging System and its Cell Analysis YT-software® (fig. 2).

The carried out experiments consistently show a much lower standard deviation contrary to the competitor (SEM ±7.5 %, data not shown). In most cases the standard deviation of cell density determinations with the NYONE® is less than 5 %. Furthermore can be seen that the measured cell density should not be less than 3.00E+5 cells/mL. Due to the small sample density of the measurement errors increase significantly. And sample densities higher than 1.5E+7, though still measurable, also increase the standard deviation again. Therefore we recommend a sample dilution depending on the expected cell density (see chapter "Procedure" point 3 (P.2)).

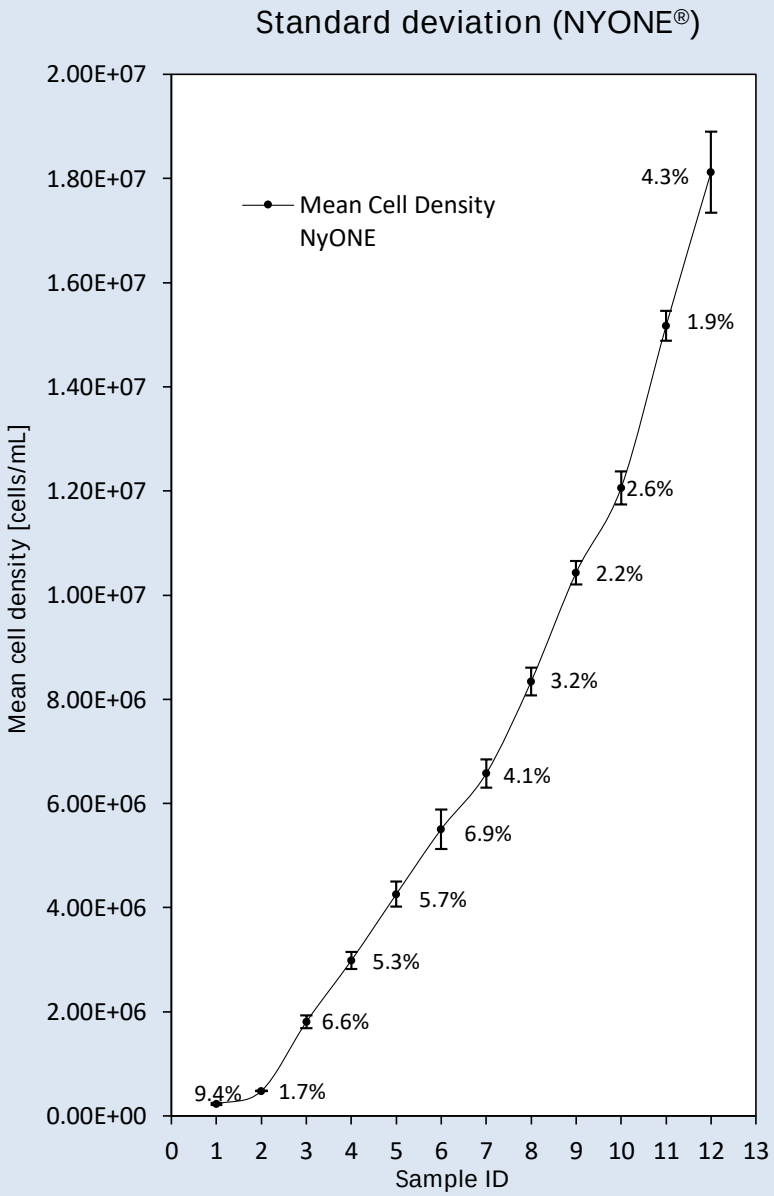
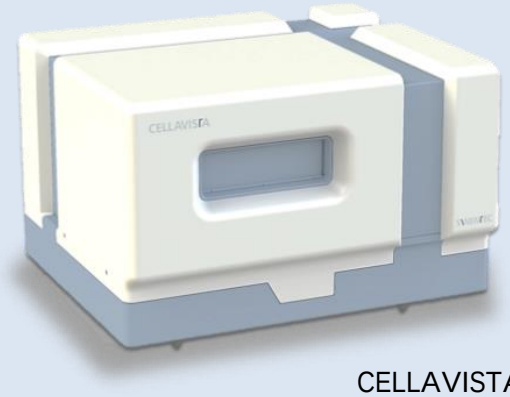


Fig. 2: Reproducibility of the NYONE® results.
Twelve samples (sample ID 1-12) of transfected CHO cells were counted with the NYONE® (green line). Cell densities between 3.00E+05 and 1.80E+07 cells/mL were used. The error-bars are shown in total cell numbers whereas the value above each error-bar indicates the standard deviation in %. Each one of the 12 samples was measured with 8 replicates (n=8).

This assay is measurable and evaluatable with all our devices and is implemented in the YT-software®.



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Acknowledgements

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