Applications

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Technical Report

Performance evaluation of white Eppendorf Polypropylene Microplates using the Promega CellTiter-Glo® Luminescent Assay

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Abstract

White-colored Eppendorf Microplates are well suited for luminescence assays because the signal intensity is maximized by reflection. Additionally, crosstalk is reduced due to the opaque material. In this Technical Report, the performance of three white Eppendorf Microplates (96/V, 96/U, 384/V) was tested using the CellTiter-Glo® Luminescent Assay (Promega). By quantifying serial dilutions of an ATP standard of 10 nM to 10 µM, it was shown that all plate types provide reliable data demonstrating the high quality of the assay system. Best sensitivity was achieved using the V-bottom shaped plates.

Introduction

Luminescent ATP assays are commonly used for quantification of viable cells in culture. If several samples have to be processed at the same time, then often the assay and the analysis are performed in a multi-well microplate format. Opaque plates are most suitable in order to prevent signal crosstalk between wells and especially white plates offer advantages in luminescence assays as they exhibit a better light reflection and increased signal compared to black plates. Black plates on the other hand are more commonly used for fluorescence assays and specifically black Eppendorf Microplates yield high sensitivity and precision in a PicoGreen® assay [1].

Eppendorf offers three types of white Microplates: two 96-well plates with either a V-bottom shape or a U-bottom shape and one 384-well plate with a V-bottom shape. All three plates are made from polypropylene, which features high temperature and chemical resistance compared to polystyrene as well as low binding capacity for nucleic acids and proteins. Careful selection of white material and an optimized well shape, combined with high quality manufacturing, is geared towards high reflection, which in turn contributes to high assay sensitivity.

The CellTiter-Glo Luminescent Cell Viability Assay (Promega) is a homogeneous method of determining the number of viable cells in culture. It is based on quantitation of the ATP present, which signals the presence of metabolically active cells. This assay is designed for use with multiwell-plate formats, making it ideal for automated high-throughput screening (HTS), and cell proliferation and cytotoxicity assays. A luminescent signal is generated which is proportional to the amount of ATP present [2]. Here, the CellTiter-Glo Assay was performed by Promega with all three types of white Eppendorf Microplates in order to evaluate the compatibility obtained with this assay system, as well as to compare the performance of each of the three different plate types with each other. Therefore an ATP standard curve consisting of a dilution series was created and several statistical parameters were calculated from the measured data.

Material and Methods

The Eppendorf Microplates 96/V, 96/U and 384/V were tested in a luminescence assay. The CellTiter-Glo Luminescent Cell Viability Assay (Promega, Cat # G7572, Lot # 268755) was used to quantify a standard curve of different concentrations of an ATP-solution.
For the experiment a 10 µM ATP solution was prepared in RPMI culture medium. 100 µl of 10 µM ATP solution contains 10^{-10} moles ATP. Thereof ten-fold serial dilutions of ATP in culture medium were created (10 µM to 10 nM). 100 µl (96-well plate) or 25 µl (384-well plate) of these dilutions of standard ATP were dispensed in the wells of the tested Microplates using an i-Pipette 96-125 Pipettor (Apricot Designs) according to the plate layout shown below (Fig. 1 a and b).

96-Well Plate layout

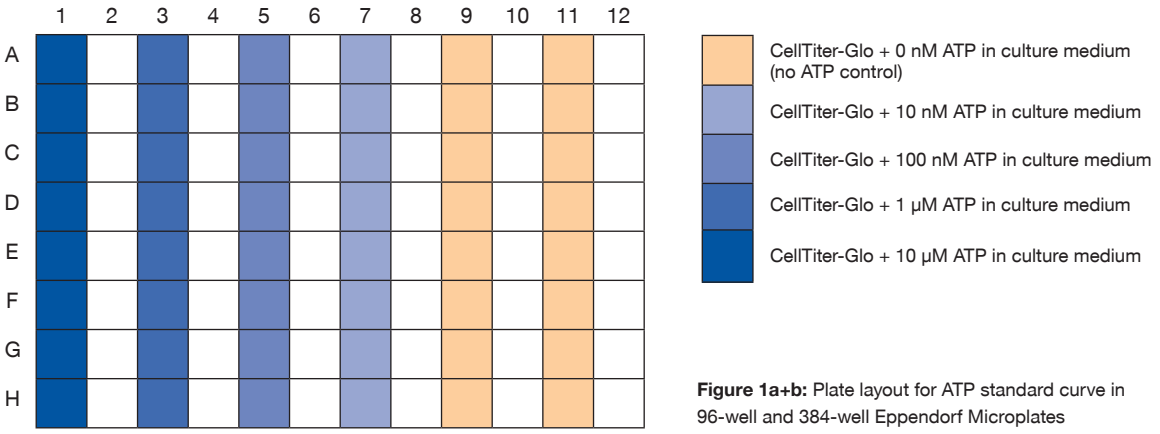
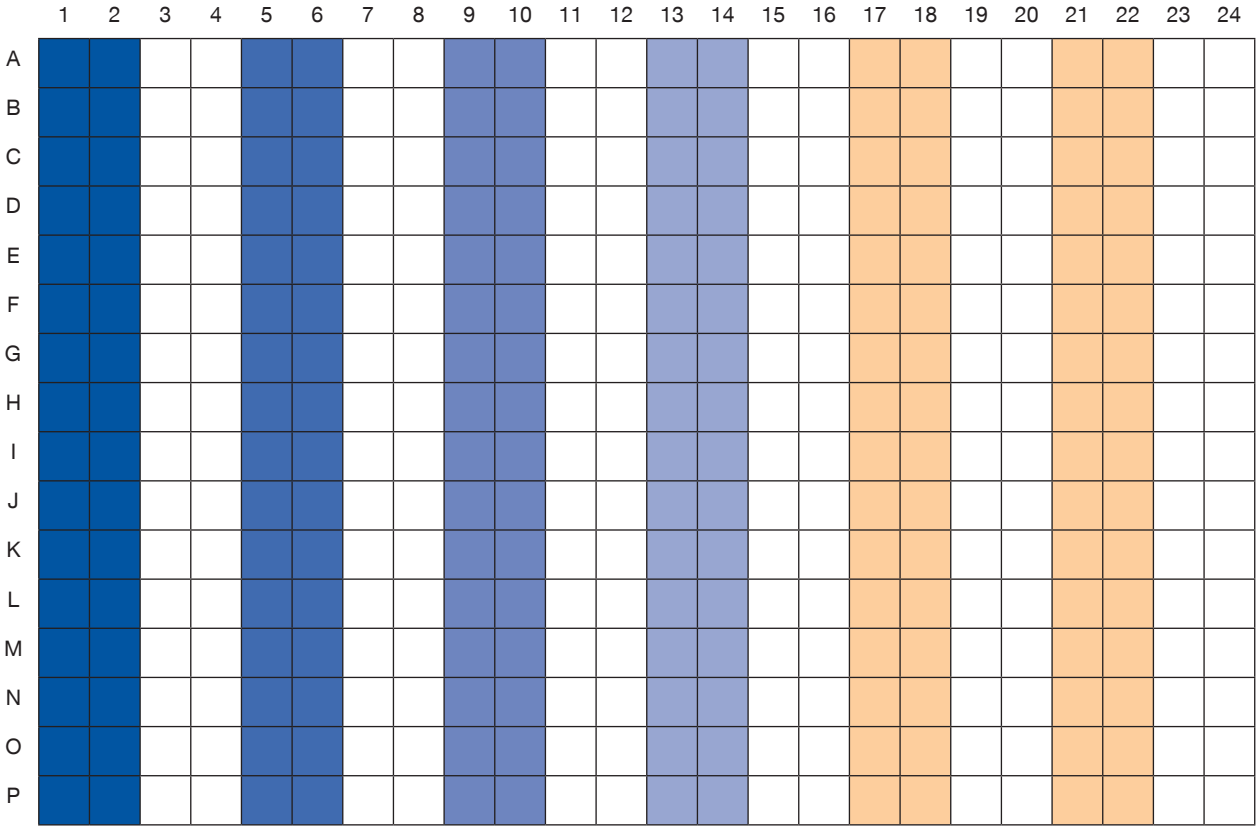


Figure 1a+b: Plate layout for ATP standard curve in 96-well and 384-well Eppendorf Microplates

384-Well Plate layout



The CellTiter Glo Reagent was prepared according to the instructions of the kit [2]. An equal volume of reagent was added to the volume of ATP standard present in each well. The plates were mixed at ~400 rpm for 2 minutes or ~1200 rpm for 384-well plates on an orbital shaker. The Microplates were allowed to incubate at room temperature for 10 minutes to stabilize the luminescent signal. Following incubation the luminescence was recorded on a Safire^{2TM} microplate reader (Tecan) using a 500 ms integration time. The data were then analyzed in a table calculation program and the following statistical parameters were calculated: average and standard deviation of luminescent signal per experimental group (RLU), coefficient of variation (%CV), limit of detection (LOD), signal to noise ratio, Z'-factor.

The linearity was determined using the linear regression of the arithmetic mean (corrected by the mean blank) derived from the corresponding replicates of one Eppendorf Microplate. The LOD is based on the mean of the negative control plus the three-fold standard deviation of this control [3]. The coefficient of variation places the variation in relation to the mean value. When calculating the ratio of signal to the standard deviation of the negative control it results in the signal to noise ratio and the Z'-factor describes the quality of an assay [4]. These parameter were used to evaluate and compare the different plate types.

Results and Discussion

The linearity of this assay in Eppendorf Plates shows the connection between concentration of the ATP standard and measured signal. The coefficients of determination equaling an $R^2 \geq 0.997$ for each plate type confirmed linearity across the entire measuring range (Figure 2). This means that ATP can be reliably determined at concentrations from 10 nM up to 10 µM.

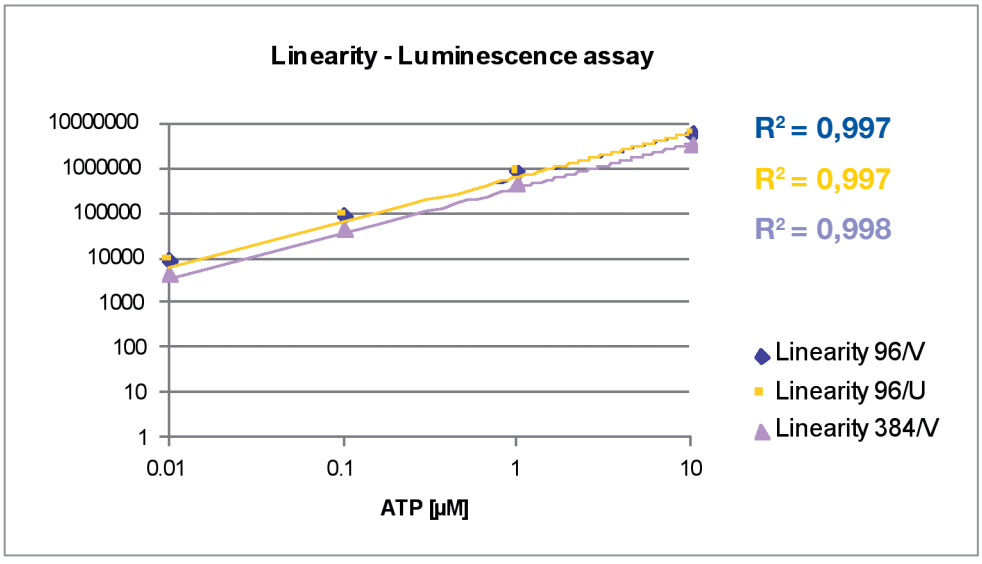


Figure 2: Logarithmic plot of data showing linearity of results for the Eppendorf Microplates 96/V, 96/U and 384/V in the range between 0.01 and 10 µM ATP.

Further criteria for evaluating the suitability of the plates are shown in Table 1. These data indicate that all plates are very well suited for the usage in luminescence assays.

The LOD is a measure for the sensitivity of an assay. The best sensitivity of 0.333 nM is observed with Eppendorf Microplate 384/V. The following parameters: coefficient of variation, signal to noise ratio and Z'-factor were analyzed for the lowest tested ATP concentration of 10 nM. Microplate 96/V shows highest precision with a CV of 2 %.

The signal to noise ratio is a measure of the relative confidence with which a signal can be considered a true signal. The higher this ratio, the better the data can be analyzed. For Microplate 384/V the highest value is shown with a ratio of 126.1, i.e. the signal can be distinguished best from background noise. The same applies to all other tested ATP concentrations (Figure 3). Values of the Z'-factor between 0.5 and 1 indicate a robust assay with reliable data, where Z'=1 represents an ideal assay [4]. The highest Z'-factor of 0.845 is achieved by Microplate 96/V.

Table 1: Statistical parameters
The best results for each parameter are shown in a darker grey.

	Microplate 96/V	Microplate 96/U	Microplate 384/V
LOD (Limit of detection)	1.113 nM	2.387 nM	0.333 nM
%CV at 10 nM	2 %	4 %	5 %
Signal-to-noise at 10 nM	39.2	18.4	126.1
Z'-factor at 10 nM	0.845	0.702	0.820

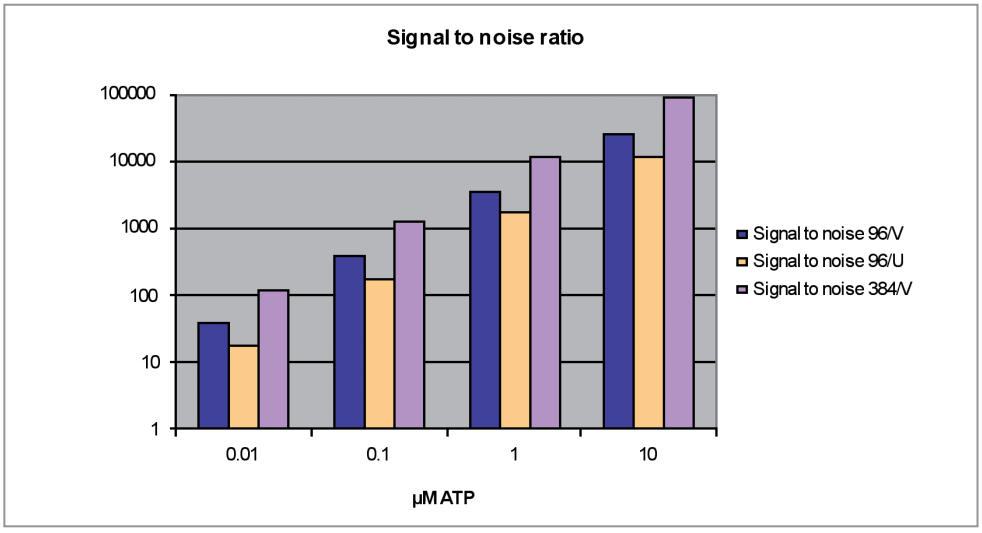


Figure 3: Signal to noise ratios at ATP standard concentrations for the Eppendorf Microplates 96/V, 96/U and 384/V

It is seen from the results that even at low concentrations of 10 nM ATP reliable data can be generated using all white Eppendorf Microplates. The lowest variation of data and best assay quality is achieved with Microplate 96/V. The V-bottom Microplate 96 showed a significantly lower limit of detection and a 2-fold higher signal to noise ratio when compared to the U-bottom Microplate 96. The very low limit of detection with Microplate 384/V which exhibits at the same time the highest signal to noise ratio indicates that this plate is especially suitable for use with low concentration of samples. When the performance of black versus white Eppendorf Microplates was compared in this assay, it was shown that black Microplates may also be used (data not shown). However, the white plates exhibited a significantly higher signal intensity.

Conclusion

White Eppendorf Polypropylene Microplates in combination with Promega's CellTiter-Glo Luminescent Cell Viability Assay exhibit a reliable and sensitive system for quantifying ATP in a wide range of concentrations. All three types of white Microplates analyzed demonstrated an excellent performance regarding assay quality, limit of detection and signal to noise ratio. The V-bottom shaped Eppendorf Microplates 96 and 384 showed the highest sensitivity in this luminescence assay and are therefore particularly suitable for low concentration samples.

References

[1] Eppendorf Application Note 203: Eppendorf Polypropylene Microplates – Highest sensitivity for fluorescence measurements in black plates (http//:www.eppendorf.com)
[2] Technical Bulletin #288 – CellTiter-Glo® Luminescent Cell Viability Assay (http//:www.promega.com)
[3] Mocak J, Bond AM, Mitchell S, Scollary G. A statistical overview of standard (IUPAC and ACS) and new procedures for determining the limits of detection and quantification: Application to voltametric and stripping techniques. IUPAC, *Pure Appl Chem* 1997, 69: 297-328.
[4] Zhang JH, Chung TD, Oldenburg KR. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* 1999; 4 (2):67-73.

Eppendorf Ordering information

Eppendorf Microplates*, 80 plates (5 bags of 16 plates each)					
Product name	**Quality**	**Well color**	**Border color**	**Order no. international**	**Order no. North America**
Microplate 96/U-PP	PCR clean	black	white	0030 601.807	951040102
Microplate 96/U-PP	PCR clean	white	grey	0030 601.572	951040145
Microplate 96/V-PP	PCR clean	black	white	0030 601.904	951040260
Microplate 96/V-PP	PCR clean	white	grey	0030 601.670	951040308
Microplate 384/V-PP	PCR clean	black	white	0030 621.905	951040481
Microplate 384/V-PP	PCR clean	white	grey	0030 621.670	951040503

*All Microplates are available with barcode and in larger packages upon request.

Promega Ordering information

Product name	Order no.
Promega CellTiter-Glo® Assay, 10 ml	G7570
Promega CellTiter-Glo® Assay, 10x10 ml	G7571
Promega CellTiter-Glo® Assay, 1x100 ml	G7572
Promega CellTiter-Glo® Assay, 10x100 ml	G7573
Promega 10 mM rATP, 500 µl	P1132

Promega

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