Applications

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Technical Report

Eppendorf Polypropylene Microplates – Highest sensitivity for fluorescence measurements in black plates

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Abstract

Black micro test plates are mainly used for fluorescence assays, since they reduce background signals and prevent cross-talk between the wells, thus rendering them ideal for analysis of even the smallest amounts of substance. Within this Technical Report, the black Eppendorf Microplate 96/V is compared to micro test plates made by other manufacturers via quantification of dsDNA using a PicoGreen® assay. The results of this experiment show that the assay performed in the Eppendorf Microplates yields the highest sensitivity and precision. Reliable data are generated even when low DNA concentrations are assayed. Thus, the Eppendorf Microplates are ideally suited for fluorescence assays.

Introduction

Today, the standard format for biochemical assays is the plate, as plates can accommodate large numbers of samples. Furthermore, ever increasing miniaturization allows a reduction in the use of both sample material and reagents.

Micro test plates are available in many variations. They are made from different materials (e.g. polystyrene, polypropylene), the bottom shape is variable (F-bottom, V-bottom, U-bottom), and apart from transparent plates, plates of white or black pigmentation are available. The choice of a suitable plate is dependent upon the nature of the reaction as well as the detection method (e.g. fluorescence, luminescence, absorption, radioactivity).

With the Microplate, Eppendorf offers an addition to the product family of Eppendorf Plates. They constitute an expansion of the Deepwell Plates, while featuring different bottom shapes. Furthermore, Eppendorf offers Microplates with black or white wells, which are especially suitable for fluorescence or luminescence measurements, respectively (Fig. 1). These plates are made from polypropylene, which features higher temperature and chemical resistance compared to polystyrene, as well as lower binding capacity for nucleic acids and proteins. Careful selection of material, combined with high quality processing, is geared towards lower autofluorescence, which in turn contributes to higher assay sensitivity.

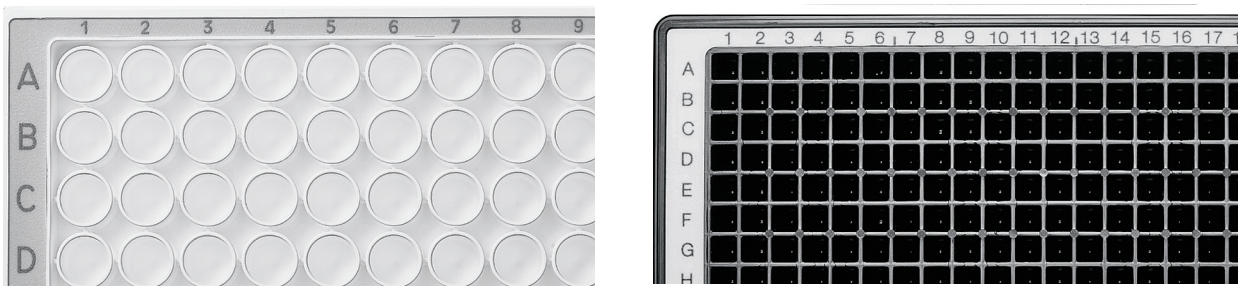

Figure 1: Black and white Eppendorf Microplate

eppendorf

The quality of an assay can be evaluated using a number of different statistical parameters. These can also be employed to evaluate the quality of data which are obtained using a specific reagent or instrument, or with the micro test plates in question. In this case, the assay is performed without sample material, but with control standards.
Here, a PicoGreen assay for dsDNA (double stranded DNA) quantification was performed in the black Eppendorf Microplate 96/V, as well as in 5 plates made by other manufacturers; 3 of which are made from polypropylene and two are made from polystyrene. The parameters "limit of detection", "signal to noise ratio" and "Z-factor" are derived from the measurements of different standard DNA concentrations, and thus the quality of the different plates used in this assay is evaluated.

Statistical parameters

Signal: Measured value, corrected by background.

Background: Average values of negative controls.

Signal to noise ratio: Calculated from the ratio of signal to the standard deviation of the negative control. It is a measure of the relative confidence with which a signal can be considered a true signal, i.e. it is distinguishable from background noise.

Standard deviation and coefficient of variation (%CV): Used to describe the precision of the assay. The standard deviation reports the scatter of the measured values around the mean, whereas the coefficient of variation places the scatter in relation to the mean value.

Limit of detection (LOD), limit of quantification (LOQ) [1]: Are a measure for the sensitivity of the assay. The lowest detectable concentration is the LOD (limit of detection), which is based on the mean of the negative control plus the threefold standard deviation of this negative control. The LOQ (limit of quantification) is the smallest quantifyable concentration based on the mean of the negative control plus the tenfold standard deviation of this negative control.

Z-factor [2]: Describes the quality of an assay. For assays which are in principle evaluable, the Z-factor assumes a dimension free value between 0 and 1. Values between 0.5 and 1 describe a robust assay with reliable data, where Z=1 stands for the ideal assay. In the case of negative values, the assay is non-evaluable. Apart from the dynamic region, the calculation of the Z-factor also encompasses the scatter of all measured values.

Materials and Methods

The black micro test plates listed in table 1 were used. Apart from polypropylene plates, plates made from polystyrene were tested; one of these was made from material deemed to have low binding capacity for biomolecules.

Name	Material	Bottom shape
Eppendorf Microplate 96/V	Polypropylene	V-bottom
Competitor N_PP	Polypropylene	V-bottom
Competitor G_PP	Polypropylene	V-bottom
Competitor C_PP	Polypropylene	U-bottom
Competitor G_PS	Polystyrene (low binding)	V-bottom
Competitor C_PS	Polystyrene	U-bottom

Table 1: Plates tested

The Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen) was used to quantify different concentrations of dsDNA. To this end, several dilutions of a 100 µg/ml solution of dsDNA (DNA is provided in the kit) were generated in 1 x TE buffer to obtain different standard concentrations. 100 µl of each concentration were filled into 6 wells along with 100 µl of PicoGreen reagent (diluted 1:200 in 1 x TE buffer). The wells thus contained the following final DNA concentrations: 25 ng/ml, 10 ng/ml, 2.5 ng/ml, 500 pg/ml, 250 pg/ml, 125 pg/ml, 62.5 pg/ml, 12.5 pg/ml. As a negative control, 100 µl TE buffer and 100 µl PicoGreen reagent were pipetted. The plate was incubated for 3 min at room temperature, protected from light. Subsequently, the fluorescence signals were measured in a Safire²™ (Tecan) set to an excitation wavelength of 485 nm and an emission wavelength of 520 nm. The data were then analyzed in a table calculation program.

Results and Discussion

Based on the measured data, the following statistical paramenters were calculated: coefficient of variation (%CV), limit of detection (LOD), signal to noise ratio, Z-factor (Fig. 3-6). These were used to compare the assays performed in the different plates in order to evaluate the quality of the plates.

The linearity of this method, which shows the connection between concentration and measured signal, was determined using the linear regression of the arithmetic mean derived from the 6 replicates for the Eppendorf Microplate. The coefficient of determination of $R^2 = 0.9998$ confirmed linearity across the entire measuring range (Fig. 2).

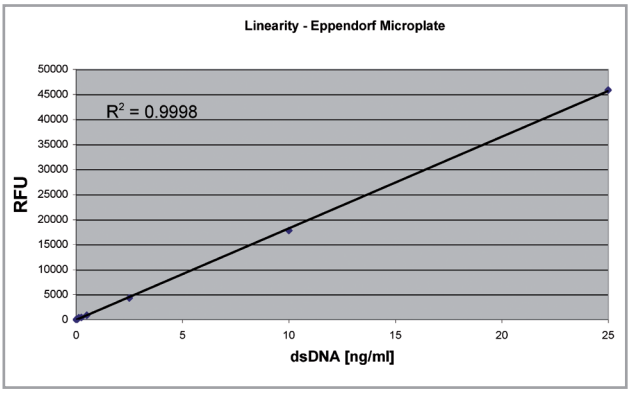

Figure 2: Linearity of results for the Eppendorf Microplate 96/V in the range between 12.5 pg/ml and 25 ng/ml dsDNA

The coefficients of variation of multiple determinations enable a statement about the precision with which a plate can be measured. To this end, the coefficients of variation of the measured concentrations 12.5 pg/ml – 25 ng/ml, as well as the negative control, were calculated and averaged. Figure 3 shows that the Eppendorf Microplates produce the lowest coefficient of variation and thus offer the best precision across the measured region compared to other plates tested.

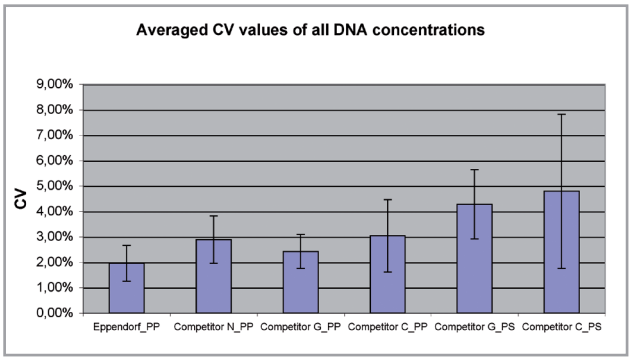

Figure 3: Average of the coefficients of variation for the measured region 0 ng/ml, 12.5 pg/ml - 25 ng/ml dsDNA, including the standard deviations for the different micro test plates.

The limit of detection is one criterion which quantifies the sensitivity of an assay. As evident from figure 4, this limit is 4 to 28 times higher for the competitors' plates than the 29 pg/ml dsDNA measured for the Eppendorf Microplate. Even the user manual for the PicoGreen Assay specifies a considerably higher limit of detection (250 pg/ml dsDNA) for use in plates and plate readers.

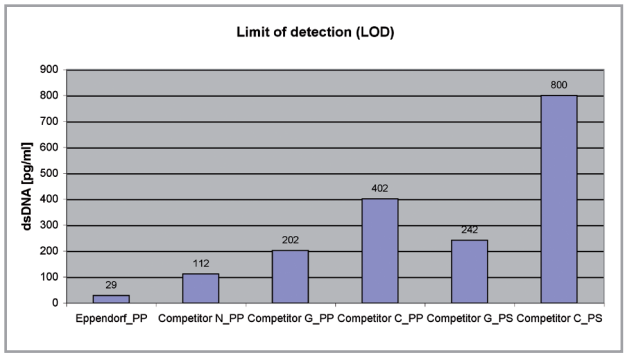

Figure 4: Limit of detection for dsDNA measured in different micro test plates, expressed in pg/ml

A further indicator for the quality of an assay is the signal to noise ratio. The higher this ratio, the better the data can be analyzed. The signal to noise ratio at a dsDNA concentration of 2.5 ng/ml is illustrated in figure 5, as this concentration has been shown to fall above the detection limit for all plates tested. Here, the Eppendorf Microplate achieves the highest value, i.e. the signal rises the farthest above the background noise. The signal to noise ratio of the Eppendorf Microplate is 4 to 28 times higher than the competitors' plates (best competitor/worst competitor, respectively).

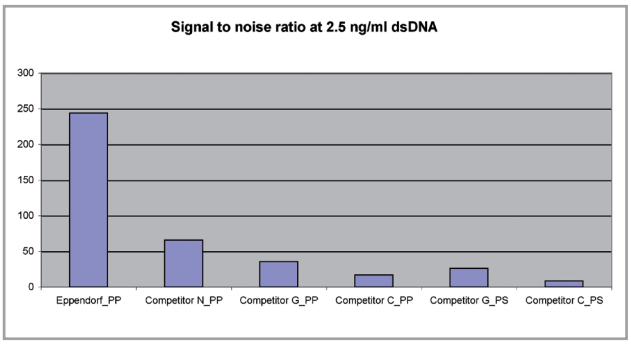

Figure 5: Signal to noise ratio at a concentration of 2.5 ng/ml dsDNA for different micro test plates

It is customary to employ the Z-factor in the evaluation of high throughput assays, since it gives an indication of the efficiency of an assay. Here it is used, if for a comparatively low number of measurements, in order to illustrate data quality, especially at lower concentration levels. In figure 6 the Z-factors for the concentrations up to 5 ng/ml dsDNA are plotted, albeit showing only the positive values, as a measurement area with a negative Z-factor cannot be evaluated. In addition, a horizontal line indicates a Z-factor of 0.5; values above 0.5 are indicative of a robust assay [2]. This parameter confirms that when Eppendorf Microplates are used, reliable data are generated even at low concentrations, while for competitors' plates a considerably higher concentration is necessary to produce data of equal quality. The difference is especially obvious between plates made from polypropylene compared to plates made from polystyrene.

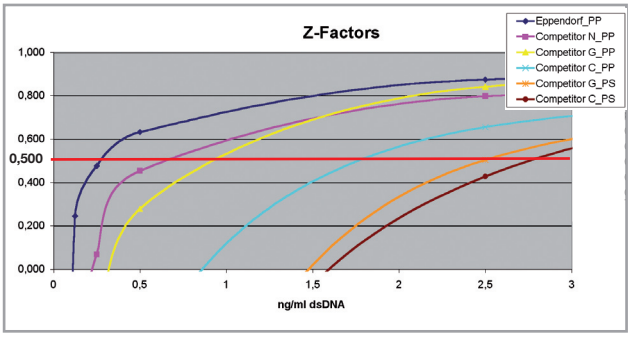

Figure 6: Z-factors for the Eppendorf Microplate as well as 5 plates made by other manufacturers. The red line indicates a Z-factor of 0.5, which is the benchmark for robust assays.

Conclusion

The results confirm that the PicoGreen assay achieves its highest efficiency when black Eppendorf Microplates 96/V are used as compared to plates made by other manufacturers. The data are very homogeneous across the measured spectrum, and the sensitivity achieved is higher than specified in the kit manual. Especially plates made from polystyrene yield considerably worse results, which can possibly be related to the increased binding affinity of this material for DNA molecules. One polystyrene plate, whose manufacturer claims low binding to biomolecules, produces only marginally better quality data.

Eppendorf Microplates are of high quality and very well suited for fluorescence assays due to the optimized production process, combined with carefully selected materials. Extremely low sample concentrations can be quantified reliably using Eppendorf Microplates.

References

[1] 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.

[2] 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.

Ordering information

Eppendorf Microplates*, 80 plates (5 bags of 16 plates each)					
Description	**Quality**	**Well color**	**Border color**	**Order no. international**	**Order no. North America**
Microplate 96/F	PCR clean Sterile	clear	white	0030 601.106 0030 602.102	951040005 951040021
Microplate 96/U	PCR clean Sterile	clear	white	0030 601.203 0030 602.200	951040048 951040081
Microplate 96/U	PCR clean	black	white	0030 601.807	951040102
Microplate 96/U	PCR clean	white	grey	0030 601.572	951040145
Microplate 96/V	PCR clean Sterile	clear	white	0030 601.300 0030 602.307	951040188 951040227
Microplate 96/V	PCR clean	black	white	0030 601.904	951040260
Microplate 96/V	PCR clean	white	grey	0030 601.670	951040308
Microplate 384/F	PCR clean Sterile	clear	white	0030 621.107 0030 622.103	951040341 951040383
Microplate 384/V	PCR clean Sterile DNA LoBind, PCR clean Protein LoBind, PCR clean	clear	white	0030 621.301 0030 622.308 0030 623.304 0030 624.300	951040421 951040464 951040546 951040589
Microplate 384/V	PCR clean	black	white	0030 621.905	951040481
Microplate 384/V	PCR clean	white	grey	0030 621.670	951040503

*All Microplates are available with barcode upon request.

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