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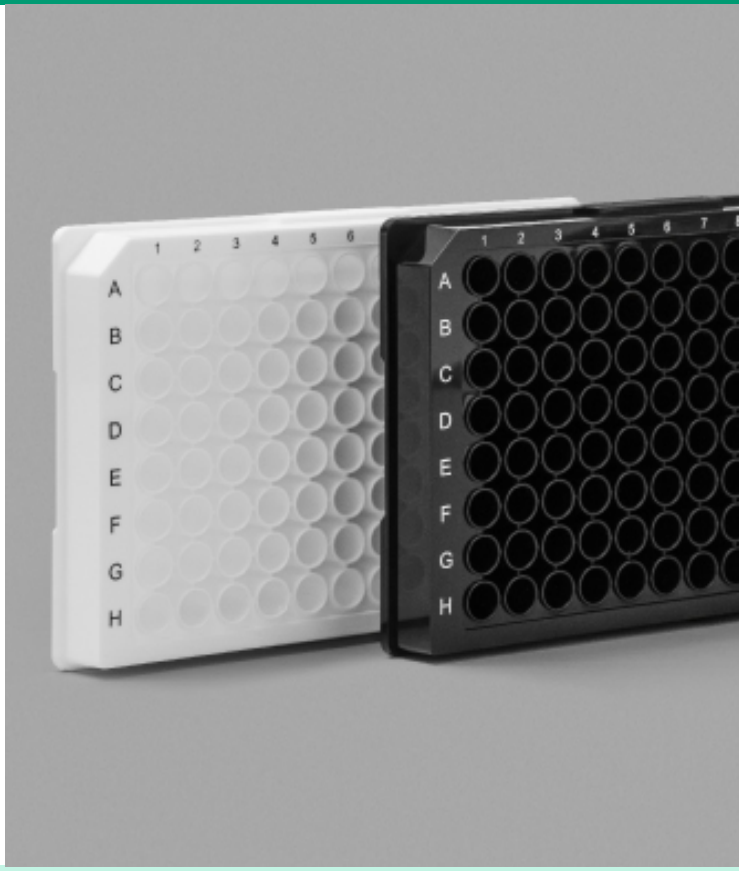
Product Characterization

Green Elephant® 96-well microPLAte flat-bottom, black/white non-sterile

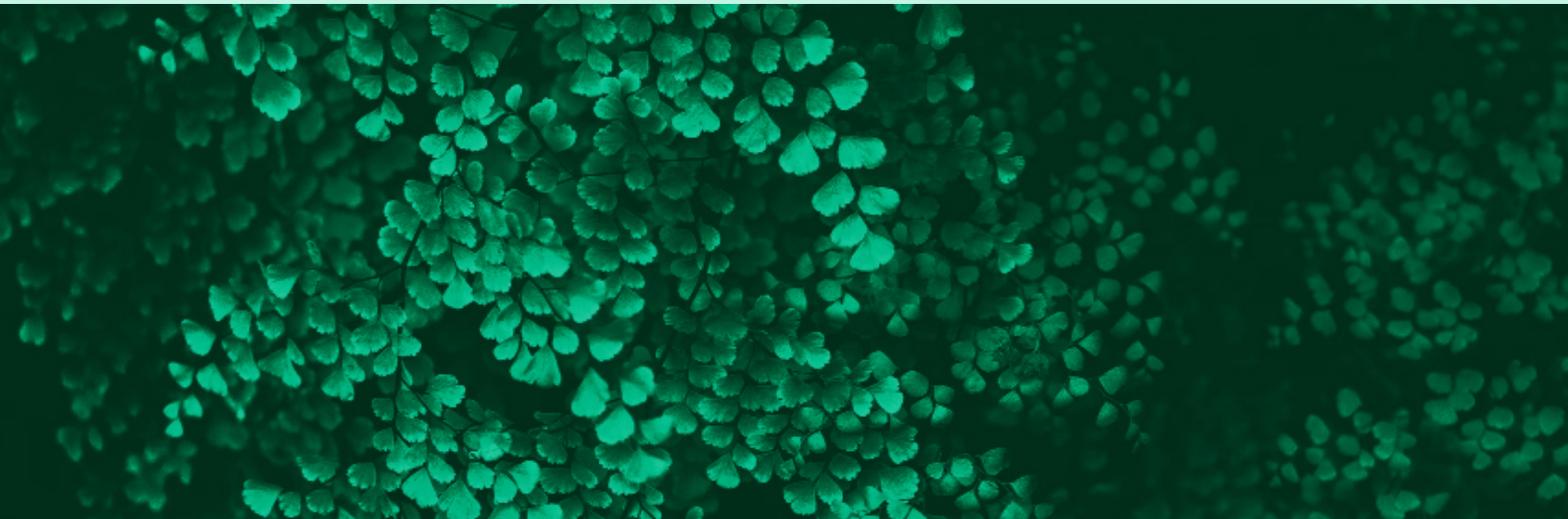


Fluorescence and Luminescence, Chemical Stability, Compatibility

1. Introduction

This report presents the characterization of a black and white Green Elephant® 96-well microPLAte made from bioplastic polylactic acid (PLA). While a fluorescence assay was performed to assess the performance of the black plate, a chemiluminescence assay was conducted for the white plate.

The results obtained are used to classify the data and make a statement about the applicability of the plates.



2. Statistical analysis

To evaluate the assays, statistical parameters such as signal-to-noise ratio, coefficient of variation, limit of detection, limit of quantification, and z-factor are determined. Table 1 displays the parameters, their definitions, evaluations, and calculations.

Table 1: List of statistical parameters used to evaluate the assays, including definitions, calculations, and evaluations.
With MV = mean value, SD = standard deviation, and NC = negative control.

Parameter	Definition	Evaluation
Signal-to-noise ratio (SNR)	Measure of the relative certainty that a signal can really be regarded as such and is not confused with background noise. Calculation: $$SNR = \frac{Signal}{SD_{NC}}$$	The higher the ratio, the better signals can be distinguished from noise and the better the data can be analyzed.
Coefficient of variation (%CV)	Describes the precision of an assay. The coefficient of variation indicates the dispersion of the values in relation to the mean value. Calculation: $$\%CV = \frac{SD_{signal}}{MV_{signal}} * 100$$	The lower the percentage coefficient of variation, the more precise the assay.
Limit of detection - LOD	Measure for the sensitivity of the assay, describing the lowest detectable concentration. Calculation: $$LOD = MV_{NC} + 3 * SD_{NC}$$ (This det then converted into concentration using linear regression.)	The lower the limit, the more sensitive the assay.
Limit of quantification - LOQ	Measure for the sensitivity of the assay, describing the lowest concentration that is still quantifiable. Calculat $$LOQ = MV_{NC} + 10 * SD_{NC}$$ (This determines the signal, which is then converted into concentration using linear regression.)	The lower the limit, the more sensitive the assay.
Z-factor	Indicates the quality of the assay. The z-factor can be a maximum of 1. Calculat $$z - factor = 1 - \frac{3 * (SD_{signal} + SD_{NC})}{\lvert MV_{signal} - MV_{NC} \rvert}$$	≥ 0,5 to 1 → good assay < 0,5 → bad assay ≤ 0 → signals and negative control overlap too much. The assay cannot be used in this range.

3. Fluorescence assay - black 96-well microPLAte

Fluorescence assays are commonly conducted in black 96-well microPLAtes. Therefore, a Quant-iT™ PicoGreen™ dsDNA assay was performed to evaluate the suitability of the plate for the fluorescence assay. A standard curve of different DNA concentrations was recorded, and statistical parameters were evaluated based on the measured relative fluorescence.

DNA concentrations ranging from 100 ng/mL to 0.0625 ng/mL, as well as a negative control, were prepared and measured using the Quant-iT™ PicoGreen™ dsDNA Reagent with an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Figure 1 displays the measured fluorescence signals in the black 96-well microPLAte.

The coefficient of determination of 0.9999 confirms a linear relationship between the concentration and the resulting fluorescence signal. Statistical parameters were used to examine assay quality and the suitability of the black 96-well microPLAte. The limit of detection is 0.6 ng/mL, comparable to available data, indicating the ability to detect even low concentrations of DNA. The limit of quantification is typically higher than the limit of detection, in this case the LOQ is 2.4 ng/mL.
The assay's precision can be described using the coefficient of variation. To compare it, the mean coefficient value was calculated over the entire measuring range. The coefficient of variation for the black 96-well plate made of PLA is 4.63 ± 1.81 %, which is in the same range as other black 96-well plates, indicating equal assay precision.
The signal-to-noise ratio at a concentration of 2.5 ng/mL in the black microPLAte is 45 ± 17, which is higher than that of other available PS-derived products, indicating good assay quality.

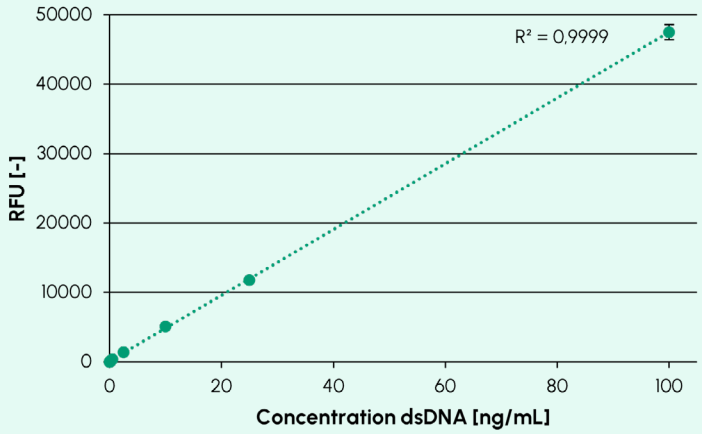


Figure 1: Fluorescence signals of the different dsDNA concentrations [ng/mL] in the black 96-well microPLAte. The linear trend line, including the coefficient of determination (R^2), is also shown. n=3.

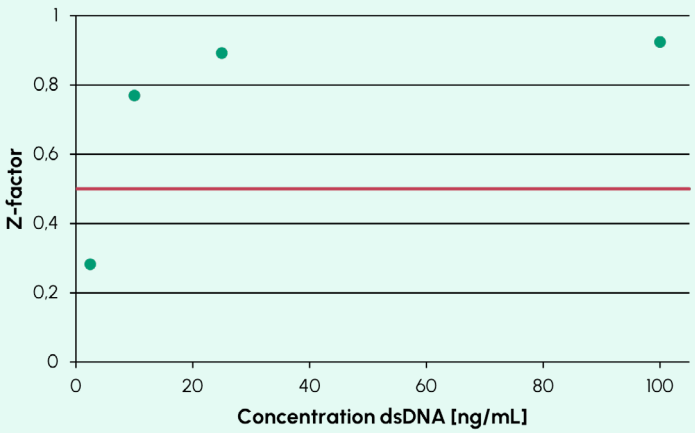


Figure 2: Z-factors of the dsDNA concentrations above the limit of quantification. The red line indicates a z-factor of 0.5.

The z-factor is the final parameter determined and is useful for evaluating the performance of high-throughput assays. Assays with values above 0.5 are considered robust and of high quality. However, for values between 0.5 and 0, the quality of the assay is reduced. The z-factors for concentrations above the limit of quantification of the black 96-well microPLAte are shown in Figure 2.

4. Chemiluminescence assay - white 96-well microPLAte

The CellTiter-Glo® assay was used to determine various ATP concentrations in the white 96-well microPLAte. The results of the assay and the use of the white 96-well microPLAte made of PLA for this purpose are subsequently discussed. Luminescence signals resulting from ATP concentrations of 10 µmol/L, 1 µmol/L, 0.1 µmol/L, and 0.01 µmol/L were statistically analyzed. The dilutions and a negative control were added to the well plates along with the CellTiter-Glo® Reagent. The luminescence signal was measured with an integration time of 500 ms.

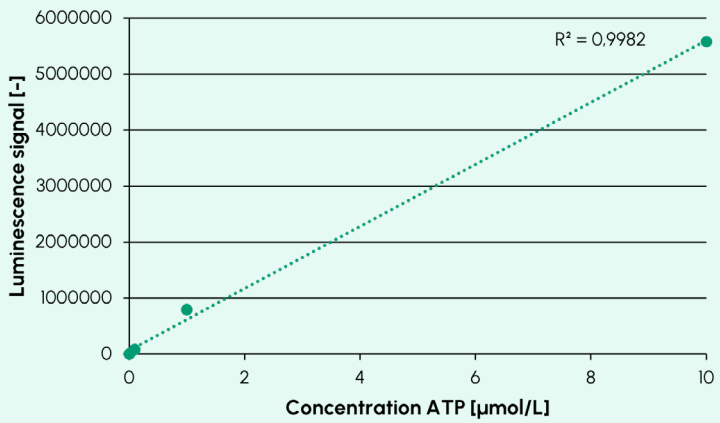


Figure 3: Luminescence signal of the different ATP concentrations [µmol/L] in the white 96-well microPLAte. The linear trend line, including the coefficient of determination (R^2), is also shown. n=3.

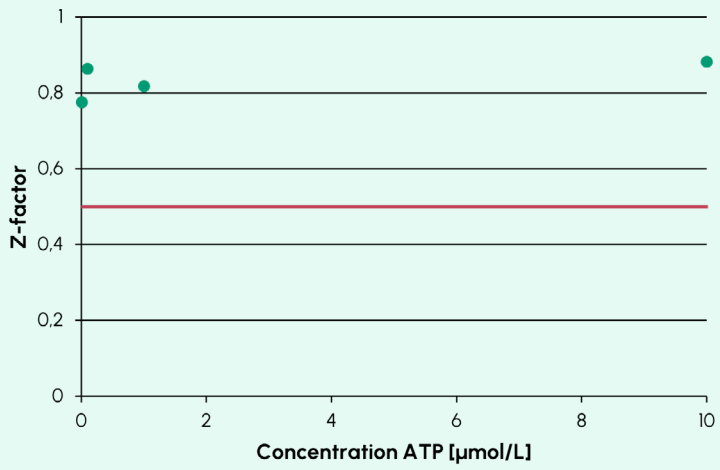


Figure 4: Z-factor of ATP concentrations above the limit of quantification. The red line marks a z-factor of 0.5.

Figure 3 illustrates the linear relationship between ATP concentration and luminescence signal with a coefficient of determination of 0.9982. The limit of detection and limit of quantification are both calculated to be below 0 µmol/L.

The percentage coefficient of variation averaged over the entire measuring range is 5.03 ± 0.89 %. Lower coefficients of variation indicate higher accuracy. Even at the lowest measured concentration of 0.01 µmol/L, the %CV is 5.7 %, which falls within a low range, demonstrating good performance. The signal-to-noise ratio determines how well signals can be distinguished from background noise; a higher ratio means better distinction. At a concentration of 0.01 µmol/L, a signal-to-noise ratio of 213 ± 35 was observed, indicating excellent performance of the white microPLAte.

The z-factor of the assay was above 0.5 over the entire measurement range, showing a robust and qualitative assay (Figure 4).

5. Compatible assays

To evaluate applicability, functionality, and performance, a series of analytical assays was conducted across all three Green Elephant® 96-well microPLAte types (clear, black, white). MicroPLAtes are suitable for use in manual operations, standard laboratory instruments including plate readers and robotics platforms. Table 1 provides an overview of analytical assays for which Green Elephant® microPLAtes are considered suitable based on current experience.

Table 1: Analytical assays for which Green Elephant® microPLAtes are considered suitable based on current experience.

transparent	black	white
✔ Colorimetric cell viability ✔ Enzyme activity assays ✔ ELISA ✔ Immunoassays ✔ Nucleic acid quantification ✔ Protein quantification ✔ Nucleic acid purification ✔ Metabolic substrate quantification ✔ Cell & colony visualization ✔ Cell Imaging ✔ Cell dilution and staining (trypan blue) ✔ Sample dilution ✔ Sample storage ✔ Flow cytometry sample prep ✔ PCR prep	✔ Fluorescent viability assays ✔ Fluorescent enzyme activity assays ✔ Fluorescent DNA/RNA quantification ✔ Fluorescent ROS, membrane potential & ion flux studies ✔ Fluorescent reporter gene systems ✔ Fluorescent protein tags ✔ Fluorescent intracellular pH & environment probes	✔ Luminescent cell viability assays ✔ Luminescent enzyme activity assays ✔ Chemiluminescent assays ✔ Luminescent ADME/mechanism of action studies ✔ Luminescent apoptosis, necrosis & cell health pathways ✔ Luminescent ROS, inflammation & receptor binding, protein-protein studies

6. Additional testing
(chemical stability, temperature resistance, and centrifugability)

Based on the acquired results, both the white and black Green Elephant® 96-well microPLAtes are viable alternatives to the polystyrene versions. The data show that the microPLAtes perform well. Furthermore, the Product Characterization Document for the clear Green Elephant® 96-well microPLAte provides additional information on its temperature and chemical stability, its suitability for DNA and protein assays, and its compatibility with centrifugation. These findings are directly applicable to the black and white PLAtes and are briefly summarized below.

"+" on a green background indicates the integrity of the plate.
"0" on a yellow ground displays minor optical differences.
"-" on a red background signifies that the plate cannot withstand the chemical and is heavily damaged by it.

Table 2: Overview of properties and results for the black and white 96-well microPLAte after 24h incubation compared to stability performance of PS.

Property		
Temperature stability	Between - 80 °C and + 40 °C	
Centrifugability	Up to 4,000 xg	
Chemical stability	**PLA - 24 h incubation**	**PS***
1- Butanol	+	+
Ethanol	+	+
Methanol (up to 70 %)	+	+
Acetone	-	-
Formaldehyde	+	0
Chloroform	-	-
Acetonitrile (up to 30 %)	+	-
DMSO (up to 10 %)	+	+
H_2O_2	+	+
HCl (up to 1 M)	+	+
Lactic Acid (13,544 M)	+	0
Acetic Acid (up to 10 %)	+	+
NaOH (up to 0.1 M)	+	+

* data collected and modified from the following sources:

1. Thermo Fisher, Polystyrene (PS) Labware - Chemical compatibility. Last accessed on 03.04.2025 from https://www.thermofisher.com

2. KMAC Plastics, Polystyrene - Chemical Resistance, Last accessed on 03.04.2025 from https://kmac-plastics.net

3. TPP, Dominique Dutscher - Chemical Resistance of Products, Last accessed on 03.04.2025 from https://www.dutscher.com/

7. DNase/RNase and human DNA testing

Independent external testing has confirmed that both products are free from RNase and DNase activity. Additionally, no traces of human DNA were detected. The testing was conducted by an independent, EN ISO/IEC 17025-accredited laboratory.

The detection limits for RNase, DNase, and human DNA were as following:

Table 3: Overview of tested parameters , method and detection limit for DNase, RNase and human DNA in Green Elephant® 96-well microPLAtes.

Tested parameter	Method	Detection limit/LOD	Result
RNase activity	RNA-Digestion	< 10^{-9} Kunitz-Units	no activity
DNase activity	DNA-Digestion	< 10^{-6} Kunitz-Units	no activity
human DNA	Realtime-PCR	< 2 pg/µL	not detected

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