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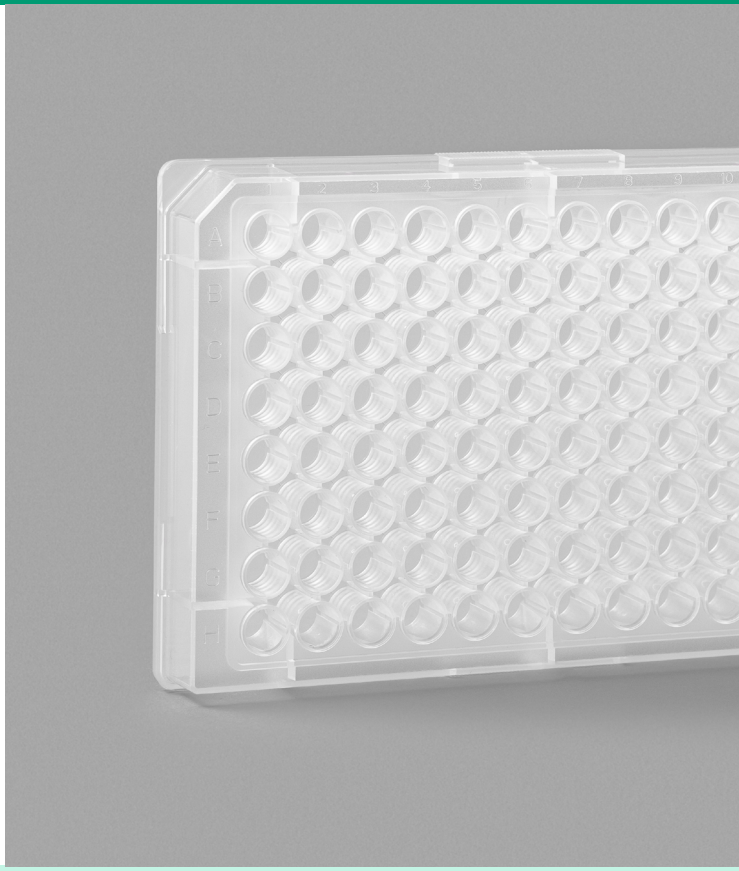
# Product Characterization

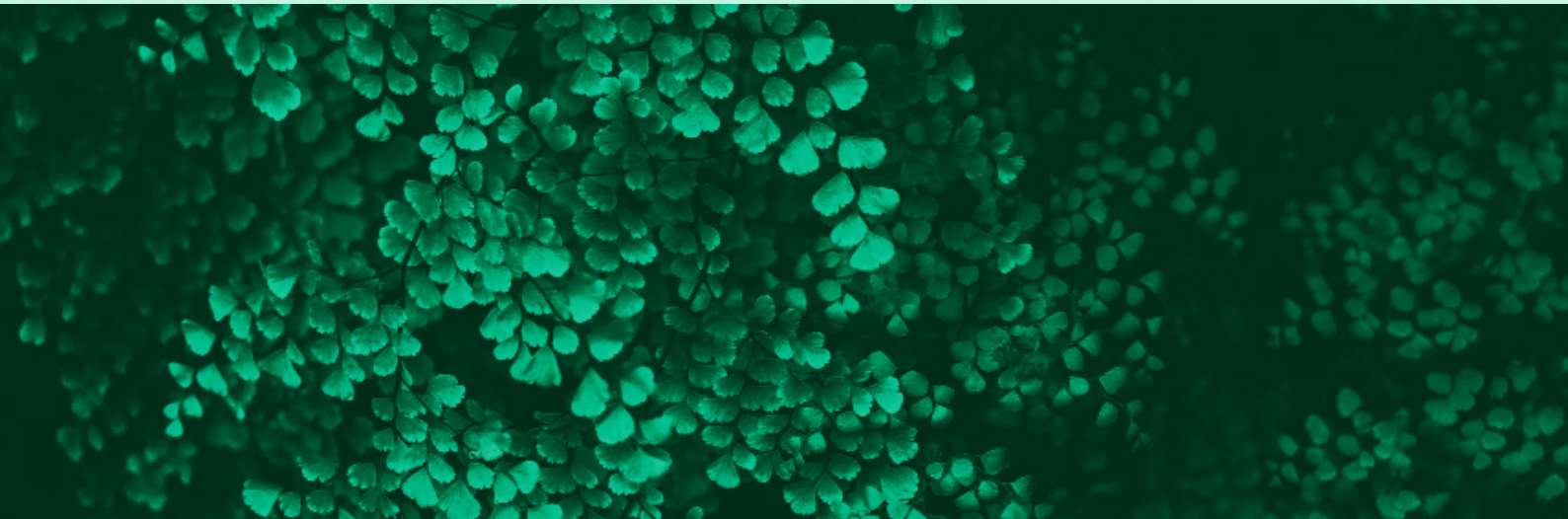
## Green Elephant® 96-well microPLAte flat-bottom, clear, non-sterile

**Absorption, Fluorescence, Protein- and DNA-Adhesion, Chemical Stability, and Biocompatibility**



## 1. Introduction

This report presents a comprehensive characterization of the Green Elephant® 96-well microPLAte made of bioplastic polylactic acid (PLA), manufactured by Green Elephant Biotech GmbH. Experiments related to absorption, fluorescence, DNA- and protein-adhesion chemical stability, and temperature resistance of the 96-well microPLAte were performed. Furthermore, the centrifugation compatibility of the 96-well microPLAte was investigated. Endotoxin and cytotoxicity assessments were conducted by an external service provider in accordance with EN ISO 10993-11 (2018) and EN ISO 10993-5 (2009) standards. To facilitate comparison, a 96-well plate made of polystyrene (PS) was used in some of the tests.



# 2. Absorption

The absorption spectra of both 96-well microPLAte (PLA) and PS 96-well plates were recorded using a plate reader, as shown in Figure 1. The mean absorbance values at wavelengths of 570 nm and 600 nm, along with their corresponding standard deviations, are shown in Figure 2.

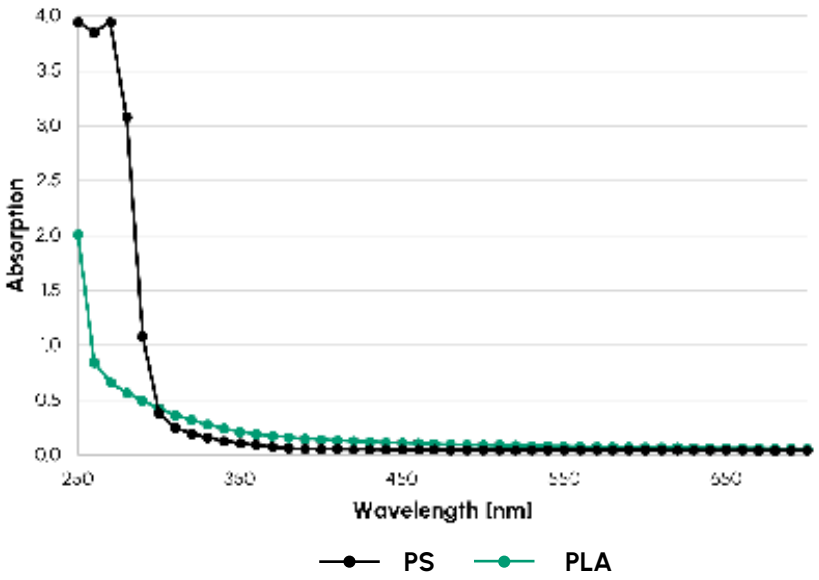



Figure 1: Comparison of the absorption spectra of the PS 96-well plates and 96-well microPLAte.

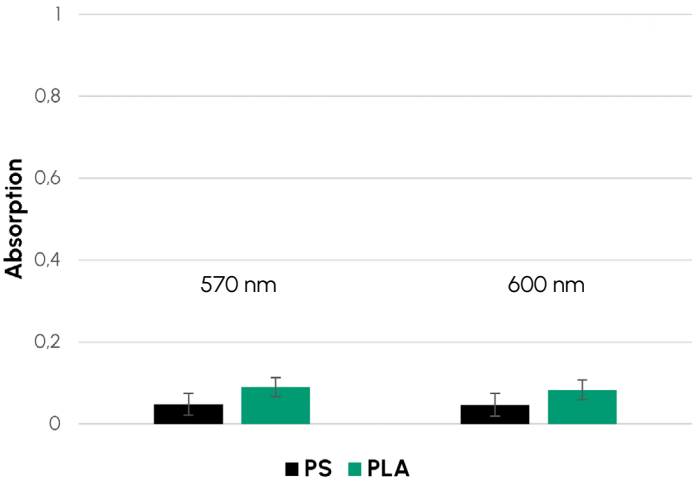



Figure 2: Mean values and the corresponding standard deviations of the absorbance at 570 nm and 600 nm for the entire PS 96-well plate and 96-well microPLAte.

# 3. Fluorescence

The fluorescence emitted by PS plates and 96-well microPLAtes, following excitation at various wavelengths, was determined using a plate reader. Excitation was carried out at wavelengths of 250 nm, 300 nm, 350 nm, 400 nm, 450 nm and 500 nm. Emission was recorded in 10 nm increments from the excitation wavelength +50 nm. The resulting fluorescence spectra are shown in Figure 3.

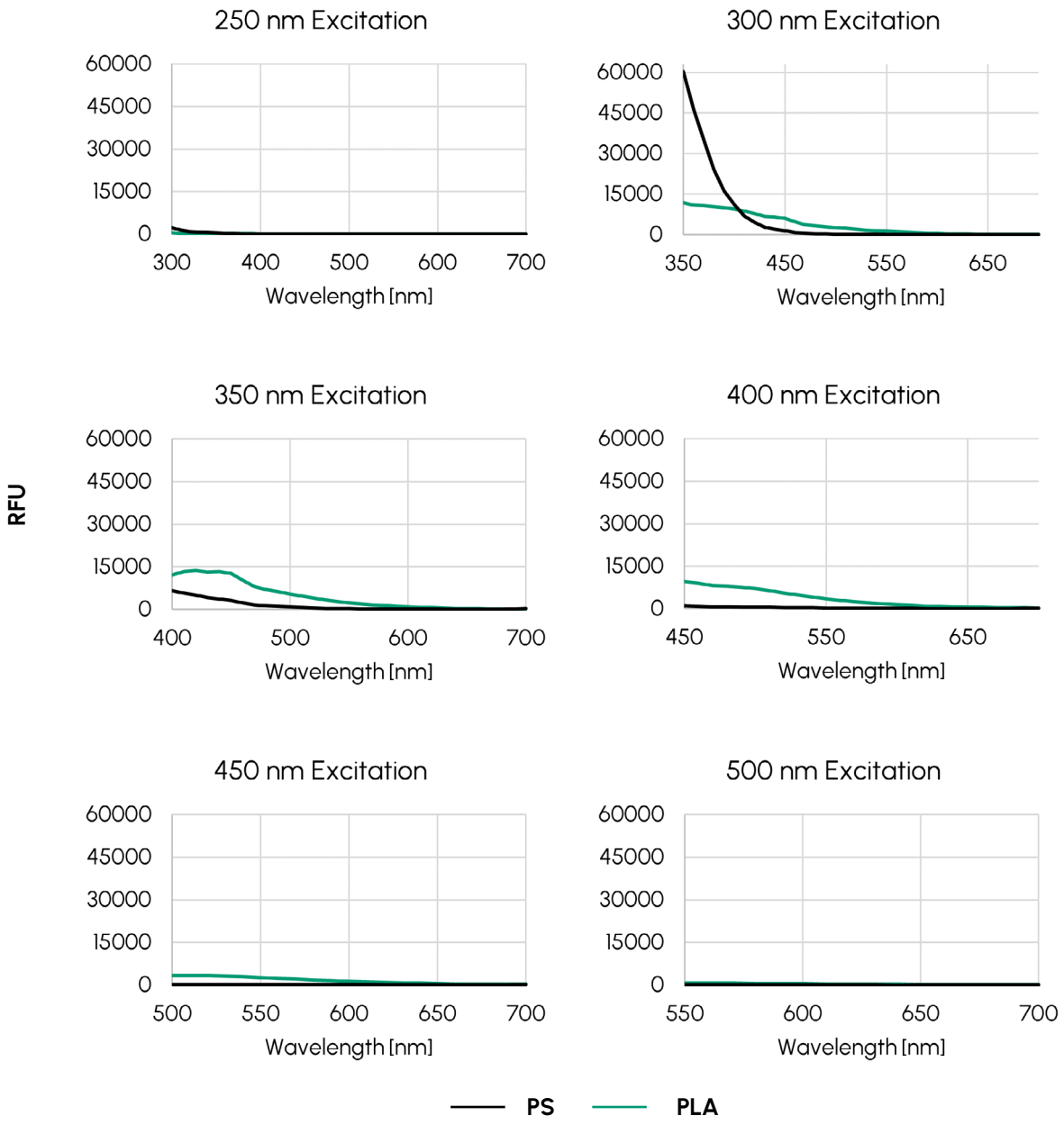



Figure 3: Comparison of fluorescence emitted by PS 96-well plates and 96-well microPLAtes after excitation at various wavelengths.

# 4. Protein-/DNA-Adhesion

## Protein-Adhesion

Following a single washing step, it was observed that only 1.4 ± 1.2 % and 2.1 ± 1.1 % of the initial 500 µg/mL BSA concentration remained on the PS plate and the 96-well microPLAte, respectively (see Figure 10). This illustrates the comparable performance between polystyrene and PLA.

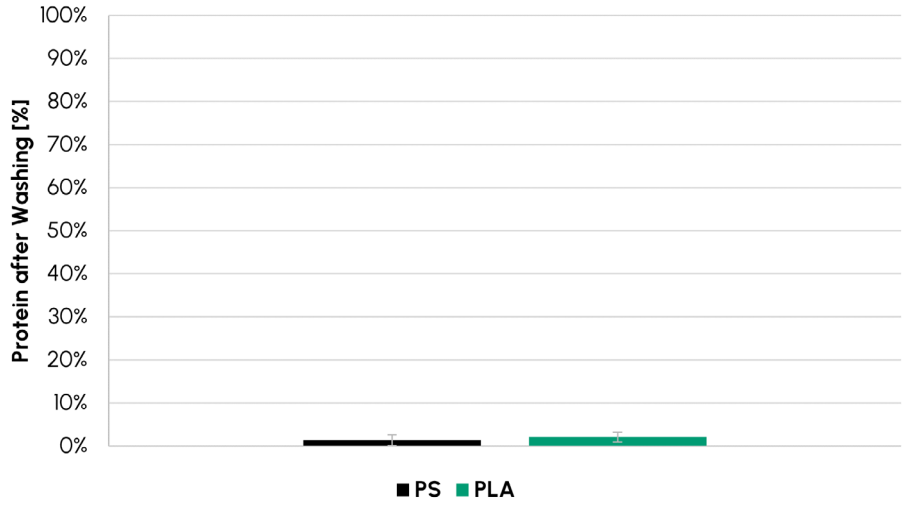



Figure 4: Amount of residual protein in the PS plates and 96-well microPLAte following a single washing step.

## DNA-Adhesion

Double stranded DNA adhesion was investigated using a standard quantification assay. Following a single washing step, Figure 5 depicts residual concentrations of 4.2 ± 0.24 ng/mL and 3.53 ± 0.35 ng/mL for the PS and PLA plates, respectively. Notably, no evidence of DNA adhesion on either the PS 96-well plate and 96-well microPLAte was determined, as the measured residues were below the limit of detection (LOD).

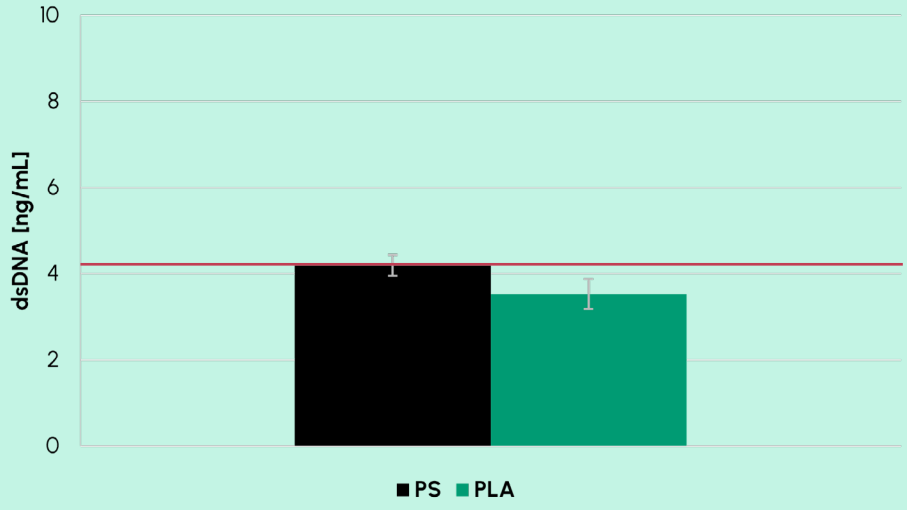



Figure 5: Concentration of residual double stranded DNA in the PS 96-well plate and 96-well microPLAte after a single washing step (LOD in red).

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

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

The chemical stability of the 96-well microPLAte was assessed after incubation for 24 h with the respective chemicals. Table 2 presents the summarized stability results, where:

"+" 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.

Furthermore, a freezing study and testing at temperatures above 37 °C demonstrates that the 96-well microPLAte can be effectively used within a temperatures range of - 80 °C and 40 °C.

Table 2: Overview of chemical stability results for PLA after 24 h incubation compared to stability performance of PS.

| Property | Results | |
|---|---|---|
| Temperature stability | Between - 80 °C and + 40 °C | |
| Centrifugability | Up to 4,000 xg | |
| **Chemical stability** | **PLA - 24 h incubation** | **PS*** |
| 1-Butanol | + | + |
| Ethanol (100 %) | + | + |
| 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. Quality control

## 7.1 Endotoxin and cytotoxicity testing

A third party laboratory determined the absence of endotoxins in the eluate of the 96-well microPLAte, following the quantitative detection of endotoxin in liquid or eluate using the Limulus amebocyte lysate (LAL) test, as per EN ISO 10993-11 (2018).

Moreover, the extract from the 96-well microPLAte exhibited a cell vitality, determined by the neutral red method, exceeding 70% compared to the blank sample. Therefore, in accordance with EN ISO 10993-5 (2009), the microPLAte is deemed non-cytotoxic.

## 7.2 DNase/RNase and human DNA testing

Independent external testing has confirmed that the product is 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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