Consumables

Low DNA-binding properties of Nunc and ABgene storage plates

Summary

- Polypropylene used by Thermo Fisher Scientific to manufacture storage plates features low DNA-binding characteristics.
- Thermo Scientific™ Nunc™ and ABgene™ polypropylene storage plates performed comparably to Eppendorf™ DNA LoBind™ plates in regard to low DNA-binding characteristics.
- Nunc and ABgene plates can be economical alternatives to the more expensive specialty plates marketed to be specifically "low DNA-binding" plates.

Introduction

Labware consumables, such as 96 deep-well plates used for storing DNA, RNA, and protein samples, are molded from polypropylene resins. Polypropylene is a hydrophobic polymer that repels the hydrophilic DNA molecules. However, the adsorption of nucleic acids and proteins onto polypropylene surfaces has been previously reported [1]. The binding of DNA to polypropylene storage plates and tubes can affect the accuracy of quantitative sample analysis and interpretation. For example, the DNA's binding to tubes and plates can further reduce the low quantities of field-collected DNA samples used in forensic, clinical, and other applications. This can result in partial or no genotype data, due to the inability to amplify a DNA sample of reduced quantity.

To avoid this scenario, some manufacturers use additives or modify the polypropylene used to manufacture storage tubes and plates, to reduce the adsorption of DNA and market them as "low DNA-binding" plates or tubes, often at premium prices [2]. Low DNA binding is a desirable feature for customers concerned about preserving their precious DNA samples. However, there is no standardized system for classifying plastic consumables based on their DNA-binding properties that would substantiate the "low DNA-binding" claims. Without a standardized system, customers cannot make an informed decision based on whether the marketed "low DNA-binding" storage plates differ in their DNA-binding properties from storage plates that do not have this designation.

Nunc DeepWell polypropylene sample processing and storage plate

The R&D team at Thermo Fisher has worked extensively to ensure minimal DNA binding when developing their storage plates. Here we present preliminary studies that were conducted at Thermo Fisher to identify low DNA-binding polypropylene, which was used to manufacture Nunc and ABgene plates. As claims of "low DNA-binding" are not standardized, we also investigated the "low DNA-binding" properties of plates from another supplier, and compared these results with those from Nunc and ABgene plates.

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Selection of low DNA-binding polymer for storage plates

Polypropylene plates are recommended for sample processing and storage of DNA molecules because of their inherent low rate of adsorbing DNA [3]. To identify a low DNA-binding polypropylene to be used for storage plates, a preliminary study was conducted to evaluate the DNA-binding capabilities of storage plates manufactured from various polypropylene resins, using ^{32}P-labeled DNA. A total of 11 storage plates from Thermo Fisher and other manufacturers, each prepared from a different polypropylene resin and some of which were specifically marketed as being "low DNA-binding", were tested to compare which plate bound the least amount of DNA. Test samples of the polypropylene were die-cut from each of the plates to avoid differences in surface shape and volume, and placed in 50 µL of a DNA solution containing 4.5 ng of ^{32}P-labeled lambda DNA, where only one side of the test sample was exposed to the DNA solution during incubation. Following overnight incubation at 20°C and after washing with Tris-EDTA (TE) buffer, the radioactivity remaining on the test samples was measured. Results of the DNA binding to the polypropylene plates, measured by radioactivity, are presented in Figure 1.

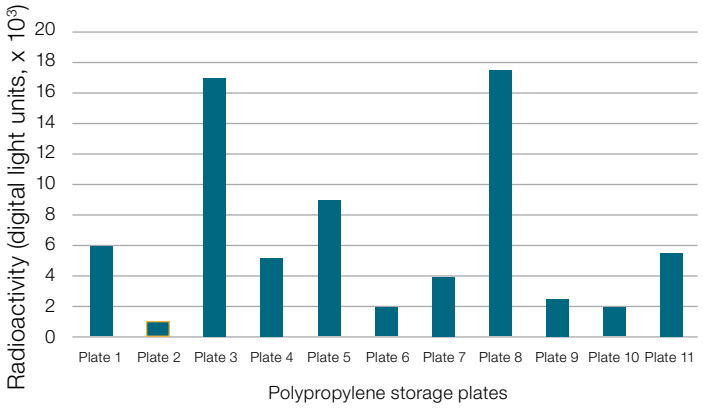

Figure 1. DNA binding on polypropylene storage plates as measured by radioactivity. Low radioactivity values indicate low DNA adsorption. Plate 2 (outlined in gold) was the best-performing storage plate with the lowest amount of DNA adsorbed.

Additionally, storage plates were tested for DNA adsorption with high ionic strength (2.5 M NaCl in TE buffer) and low ionic strength (TE buffer, 10 mM Tris-HCl with 1 mM EDTA) buffers (Figure 2). DNA adsorption to the polypropylene surface is favored by a high ionic strength buffer. However, DNA is commonly stored and used under low ionic strength buffer conditions.

Overall, the polypropylene from plate 2 had the lowest DNA adsorption properties (Figures 1 and 2), and consequently was used to develop and manufacture Nunc and ABgene storage plates.

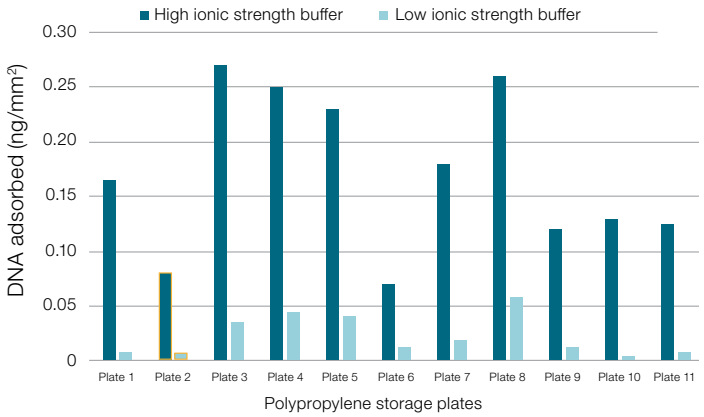

Figure 2. Adsorption of DNA (in ng/mm²) to different storage plate types under low ionic strength (TE buffer, 10 mM Tris-HCl with 1 mM EDTA) and high ionic strength (2.5 M NaCl in TE buffer) conditions. Plate 2 (outlined in gold) was the best-performing storage plate with the lowest amounts of DNA adsorbed, in the presence of both high and low ionic strength buffers.

Testing low DNA-binding plates in molecular biology applications

Thermo Fisher tests the performance of its consumable products in various workflows and applications used by its customers. For example, Thermo Scientific™ DeepWell™ storage plates are used in molecular biology applications for storing, extracting, and amplifying DNA and RNA samples. In the following experiments, DNA binding to storage plates was measured by simulating their use in a common qPCR workflow. The DNA-binding properties of Nunc and ABgene storage plates were compared to those of storage plates that are specifically marketed to be "low DNA-binding" from another supplier.

Materials and methods

The Nunc 96-Well Polypropylene DeepWell Storage Plate (Cat. No. 278743) and ABgene 96-Well Polypropylene DeepWell Storage Plate (Cat. No. AB0661) were compared to the Eppendorf™ DNA LoBind™ Deepwell Plate (Eppendorf, Cat. No. 951032808) for DNA binding.

Nunc DeepWell storage plates are frequently used for biological sample collection, sample preparation for next-generation sequencing (NGS), genomic banks, storage of DNA and siRNA, manual and high-throughput plasmid purification, storage of oligonucleotide libraries, preparation of peptide libraries, protein analysis, and drug discovery applications. ABgene DeepWell storage plates are highly cited for library preparation steps for NGS and nucleic acid extraction workflows. Nunc and ABgene plates were compared to Eppendorf DNA LoBind Deepwell plates because of the manufacturer's claim that the product provides maximum DNA recovery and reduces sample-to-surface binding without the need for surface coating, particularly when compared to products of other brands.

A common qPCR workflow was used to detect the differences in DNA adsorption between the storage plates [4]. The qPCR amplification reactions magnified differences in the DNA quantities, expressed in C_t values, due to adsorption losses. Four time points at 0 hour (negative control), 4 hours, 24 hours, and 48 hours were investigated to simulate short- and long-term storage conditions at 25°C (room temperature).

Preparing serial dilutions of the template DNA is a critical step toward reliable and accurate sample quantification by qPCR, enabling precise determination of the amplification efficiency, where adsorption of the molecules to the plastic resin is inferred from C_t values [5]. A 10-fold dilution series of human genomic DNA (gDNA) comprising three orders of magnitude (100,000 copies down to 100 copies) was prepared. Each dilution sample was split into 200 µL aliquots, transferred to a deep-well plate in quadruplicate, and incubated at room temperature. The validated Applied Biosystems™ TaqMan™ RNase P Detection Assay (Cat. No. 4316831) was performed using an Applied Biosystems™ QuantStudio™ 5 Real-Time PCR System, 384-well (Cat. No. A28140). TaqMan RNase P Detection Assay performance was validated at >99% efficiency. High amplification efficiency was deemed the most important criterion for choosing this specific assay for DNA-binding experiments, as this eliminates variation in C_t values due to differences in assay efficiency and ensures that all measured variation is from gDNA binding to the tested storage plates [6]. Therefore, differences in C_t values were the result of reduced template quantities caused by DNA binding to the plates. All data means were compared by statistical analysis using Student's t-test at $\alpha = 0.05$.

Results

The average loss of DNA across all time points (0, 4, 24, 48 hr) due to DNA binding to polypropylene among Nunc, ABgene, and Eppendorf plates can be seen in Figure 3. For all tested concentrations, the mean C_t values were all comparable. Although slight variation in C_t values can be seen at each gDNA copy number, no clear trends were observed. Nunc and ABgene plates performed as favorably as Eppendorf plates. It can be concluded that there were no substantial differences among the marketed "low DNA-binding" Eppendorf plates and Nunc and ABgene plates.

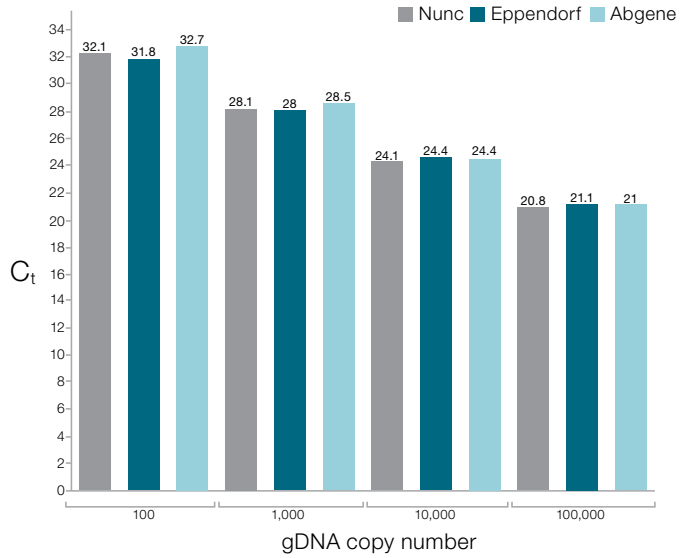

Figure 3. Mean C_t values at various gDNA copy numbers. Lower C_t values correlate to higher DNA recovery and thus lower DNA binding to the plate.

Next, we sought to evaluate how storage time can influence the amount of binding for different amounts of DNA stored and the plate type, based on the same collected data. The effects of storage time can be seen in Figure 4. Similarly, no meaningful difference in C_t values were observed within the same gDNA copy numbers among Nunc, ABgene, and Eppendorf plates at various storage times (0, 4, 24, 48 hr).

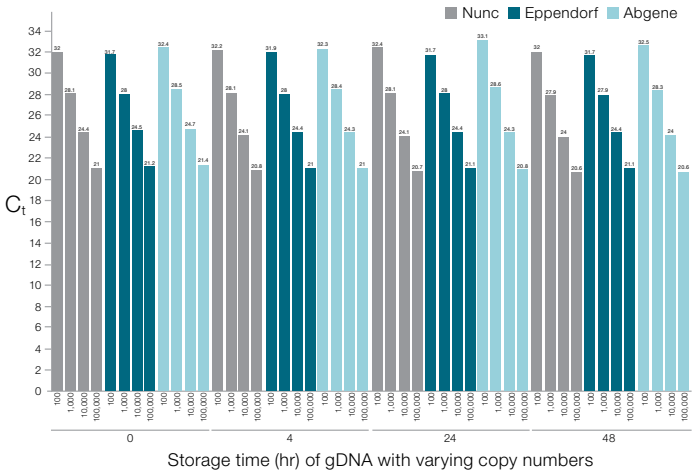

Figure 4. C_t values of gDNA with varying copy numbers, stored for different lengths of time (0, 4, 24, and 48 hr) in Nunc, ABgene, and Eppendorf plates.

Room temperature storage up to 48 hours had minimal effect on C_t for all three plate types, meaning even after storage, Nunc and ABgene plates performed comparably to the marketed “low DNA-binding” Eppendorf plates. Therefore, the DNA-binding characteristics of the storage plates can be deemed mostly negligible over time, and gDNA can be safely stored up to 48 hours in Nunc, ABgene, or Eppendorf plates.

Conclusion

Nunc and ABgene polypropylene plates provide a trusted solution for manipulating large numbers of samples for PCR, NGS, and other workflows that require low DNA-binding properties. Nunc and ABgene DeepWell storage plates are particularly convenient for storing biological specimens, including nucleic acids. Both brands of plates are made of virgin medical-grade polypropylene with low DNA-binding characteristics, which prevents sample loss from DNA binding to the storage plate. Specialty products marketed to be “low DNA-binding” are available, yet often come at a premium price. Here we demonstrated that the performance of Eppendorf DNA LoBind plates, which are marketed specifically to be “low DNA-binding”, can be matched by Nunc and ABgene polypropylene plates. Thermo Scientific polypropylene plates are effective solutions for DNA sample storage in 48-, 96-, and 384-well formats.

References

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ABgene DeepWell polypropylene sample processing and storage plate

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