

## Evaluation of **SERATEC<sup>®</sup> ZebraShield**, a commercially available nucleic acid stabilization reagent

### Introduction

The preservation of DNA at room temperature has long posed a challenge in forensic genetics, clinical diagnostics, and biobanking. Traditionally, low-temperature storage methods have been employed to ensure DNA stability over time, including refrigeration and freezing. However, these methods are not only costly but also logistically limiting, especially in fieldwork scenarios or resource-limited settings where continuous cold chains cannot be guaranteed [1–3].

Recent advances have focused on developing stabilization technologies that allow DNA to be stored at ambient temperatures without significant degradation. Despite the availability of commercial solutions, studies have highlighted various limitations, such as susceptibility to atmospheric moisture, oxidative damage, or reliance on specialized materials that may not integrate well with standard laboratory workflows [1,4].

To address some of these limitations, this application note evaluates ZebraShield, a commercially available nucleic acid stabilization reagent of SERATEC, Germany. ZebraShield is a molecular transport medium specifically formulated to preserve and stabilize DNA and RNA while simultaneously inactivating pathogens during sample collection, transport, and storage. Importantly, ZebraShield ensures nucleic acid preservation at ambient temperatures. This capacity significantly reduces the reliance on cold-chain logistics and improves accessibility for research and diagnostics conducted in remote or resource-constrained settings, including fieldwork. The reagent is compatible with various collection devices—such as swabs, blood tubes, fecal tubes, and lysis tubes—supporting its versatility across clinical, research, and field applications. This application note outlines the performance of ZebraShield in a simple sample storage experiment.

### Methods

A fresh sample of dog fecal was used for the experiment. From this sample, 150 mg portions were taken in triplicate and immediately processed to represent time point zero (T0). An additional three

sets of 150 mg portions were prepared and stored under three different conditions for a period of one week:

1. ZebraShield: Samples were immersed in ZebraShield solution.
2. Dried: Samples were left to dry under ambient conditions.
3. Moist: Samples were stored in sealed plastic tubes to maintain moisture without drying.

After one week, DNA was extracted from all samples using Quick-DNA Fecal/Soil Microbe Miniprep Kit (Zymo Research, USA), following the manufacturer's protocol. DNA quantity and quality were assessed using a qPCR assay with SYBR Green (Bio-Rad, USA) detection. Two housekeeping genes were targeted: 28S rRNA (133 bp amplicon) and cyclophilin (CY) (369 bp amplicon). The difference in amplicon length was used to evaluate DNA degradation. All measurements were conducted in triplicate to ensure reproducibility.

## Results and Discussion

Quantitative PCR analysis targeting two amplicons of different lengths (28S rRNA, 133 bp; cyclophilin, 369 bp) [5,6] was used to evaluate DNA degradation across different storage conditions. The fresh fecal samples (T0) DNA concentrations served as a baseline for comparison. After one week of storage, apparent differences emerged between the preservation methods. Samples stored in ZebraShield yielded the highest DNA concentration in the shorter and the longer amplicon. Samples stored in ZebraShield showed the highest DNA yield and lowest variation in values across all replicates, indicating efficient stabilization of nucleic acids. Amplification of short and long amplicons remained consistent, suggesting minimal degradation occurred under these conditions. In contrast, samples stored under dry and moist conditions without stabilization exhibited lower DNA yield and more significant variability in amplification efficiency.

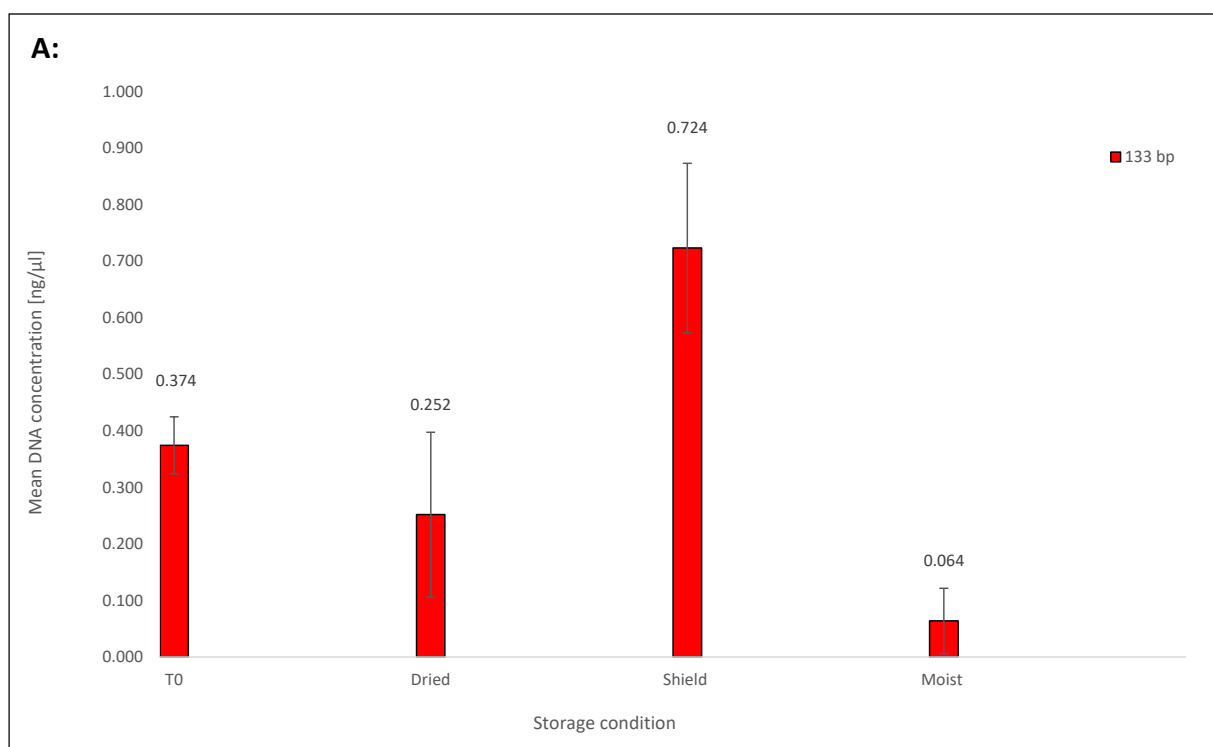
The most pronounced degradation was observed in dried and moist samples, respectively. These findings align with earlier reports that exposure to atmospheric oxygen and moisture can accelerate DNA degradation during dry storage [2].

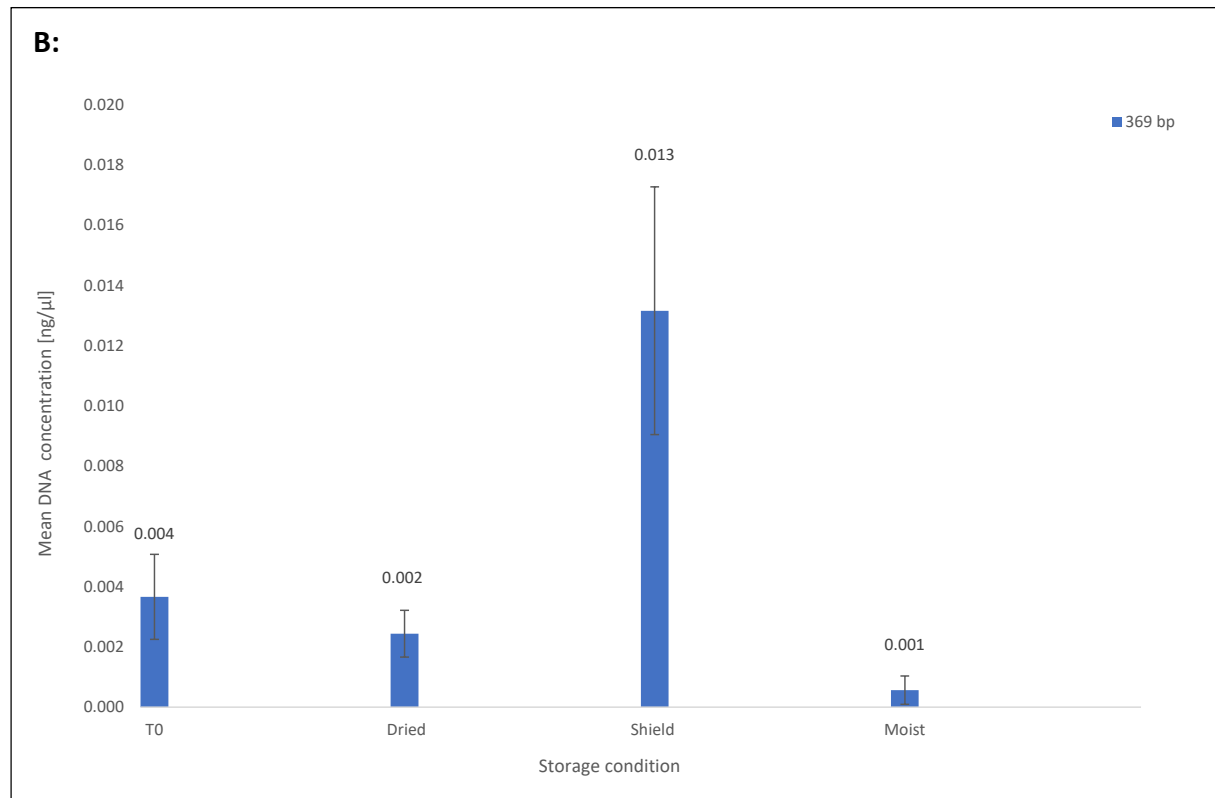
Our results confirm previous findings that dry and moist ambient conditions can negatively impact DNA stability [4], and reinforce the advantages of using chemical preservatives such as ZebraShield. Similar studies have demonstrated the importance of protecting DNA from hydrolysis and oxidation, particularly in the absence of cold storage [7,8].

Importantly, this study supports the broader utility of ZebraShield not only in clinical and molecular diagnostics but also in wildlife, forensic, and environmental research, where maintaining nucleic acid

integrity in the field is crucial. The preservation performance observed here is consistent with previous evaluations of ZebraShield in diverse sample matrices, including glacial environments and microbial communities.

Together, these findings underscore the potential of ZebraShield to enable reliable, cold-chain-independent storage of DNA in resource-limited settings without compromising downstream molecular applications.





**Figure 1.** Bar plots showing qPCR-based DNA quantification using the SYBR Green detection method. The x-axis represents the different storage conditions applied to the samples, while the y-axis displays the mean DNA concentration measured. All samples were processed in triplicate.

**A:** Amplification results for the 28S rDNA target (133 bp amplicon).

**B:** Amplification results for the cyclophilin target (369 bp amplicon), used to assess the impact of storage conditions on longer DNA fragments

## Conclusion

This application note highlights the efficacy of ZebraShield as a nucleic acid preservation reagent for room temperature storage. By demonstrating DNA stabilization under ambient conditions compared to dry or moist storage without preservatives, ZebraShield proves especially valuable in scenarios lacking refrigeration infrastructure. Its wide compatibility with various sample collection formats—such as swabs, lysis tubes, and fecal sample containers—further enhances its utility across clinical diagnostics, wildlife forensics, and environmental/conservation field research. Inactivating pathogens and protecting nucleic acids from hydrolysis and oxidative damage, ZebraShield enables reliable

sample transport and processing in diverse and often challenging environments. As demonstrated in this application note and supported by existing literature, it offers a practical, cold-chain-free solution for preserving DNA integrity across a wide range of scientific and diagnostic applications.

## References

- [1] S.E. Howlett, H.S. Castillo, L.J. Gioeni, J.M. Robertson, J. Donfack, Evaluation of DNASTable<sup>TM</sup> for DNA storage at ambient temperature, *Forensic Sci Int Genet* 8 (2014) 170–178.  
<https://doi.org/10.1016/j.fsigen.2013.09.003>.
- [2] M. Colotte, D. Coudy, S. Tuffet, J. Bonnet, Adverse effect of air exposure on the stability of DNA stored at room temperature, *Biopreserv Biobank* 9 (2011) 47–50. <https://doi.org/10.1089/bio.2010.0028>.
- [3] J. Bonnet, M. Colotte, D. Coudy, V. Couallier, J. Portier, B. Morin, S. Tuffet, Chain and conformation stability of solid-state DNA: Implications for room temperature storage, *Nucleic Acids Res* 38 (2009) 1531–1546.  
<https://doi.org/10.1093/nar/gkp1060>.
- [4] D. Coudy, M. Colotte, A. Luis, S. Tuffet, J. Bonnet, Long term conservation of DNA at ambient temperature. Implications for DNA data storage, *PLoS One* 16 (2021). <https://doi.org/10.1371/journal.pone.0259868>.
- [5] O.I. Hoffmann, A. Kerekes, N. Lipták, L. Hiripi, S. Bodo, G. Szaloki, S. Klein, Z. Ivics, W.A. Kues, Z. Bosze, Transposon-Based Reporter Marking Provides Functional Evidence for Intercellular Bridges in the Male Germline of Rabbits, *PLoS One* 11 (2016) e0154489. <https://doi.org/10.1371/journal.pone.0154489>.
- [6] M. Dawoud Al-Bader, H. Ali Al-Sarraf, Housekeeping gene expression during fetal brain development in the rat—validation by semi-quantitative RT-PCR, *Developmental Brain Research* 156 (2005) 38–45.  
<https://doi.org/10.1016/j.devbrainres.2005.01.010>.
- [7] H. Liu, Y. Gan, Y. Wu, H. Weng, P. Lei, G. Shen, Effects of different lysis buffers of nucleic acid purification kit on the stability of influenza virus RNA, *Future Virol* 9 (2014) 549–555. <https://doi.org/10.2217/fvl.14.39>.
- [8] C.B. Trivedi, C. Keuschnig, C. Larose, D.V. Rissi, R. Mourrot, J.A. Bradley, M. Winkel, L.G. Benning, DNA/RNA Preservation in Glacial Snow and Ice Samples, *Front Microbiol* 13 (2022).  
<https://doi.org/10.3389/fmicb.2022.894893>.