Stability of DNA Stored in Norgen's Saliva DNA Preservative at 55°C

W. KIM, PH.D[1] , M. EL-MOGY, PH.D[1] , AND Y. HAJ-AHMAD, PH.D[1,2]
[1]NORGEN BIOTEK CORPORATION, THOROLD, ONTARIO, CANADA
[2]CENTRE FOR BIOTECHNOLOGY. BROCK UNIVERSITY, ST. CATHARINES, ONTARIO, CANADA

INTRODUCTION

In recent years attention has been turning to the use of non-invasive samples for genetic and diagnostic analysis, including the use of saliva. The isolation of high quality DNA from saliva is not without its problems however. The number of DNA-containing cells found in saliva can vary significantly from individual to individual. Adequate amounts of saliva must therefore be collected to ensure that DNA can be extracted in an amount sufficient for testing. As enzymes that degrade DNA are found in saliva, methods must also be employed to protect the DNA in the sample. For currently available collection devices which do not employ preservative this requires that the saliva be collected into approved cryovials, held on ice and then frozen at -20°C as soon as possible. The addition of preservative to the collection vials eliminates the need to immediately process or freeze the saliva samples and allows the samples to be shipped at ambient temperature.

Norgen Biotek Corp. has developed a Saliva DNA Preservative which allows for the long-term preservation of saliva samples at ambient room temperature, making this buffer ideal for saliva storage and shipping. This buffer is available as a product on its own, and is also included with our different saliva DNA collection, preservation, storage and purification devices and kits. The Saliva DNA Preservative is an aqueous storage buffer designed for rapid cellular lysis and subsequent preservation of saliva DNA from fresh specimens. This preservative stabilizes the DNA for long-term storage at ambient temperature. Since the buffer prevents the growth of microorganisms and inactivates viruses it also allows the samples to be handled and shipped safely. The DNA subsequently isolated from the preserved samples is of a high quality and can be used directly in sensitive downstream diagnostic assays such as real-time PCR.

In addition to preserving saliva at ambient temperatures, Norgen's Saliva DNA Preservative also allows for the preservation of saliva at temperatures up to 55°C. This is very important, as these Saliva Collection and Preservation Devices may need to be shipped to and used in areas where the temperature is much warmer than ambient room temperature. Therefore, in this application note, the longterm stability of saliva DNA in Norgen's Saliva DNA Preservative at 55°C is analyzed.

METHODS AND MATERIALS

DNA Isolation

Saliva samples were collected from numerous donors and mixed, and then an equal volume of Norgen's Saliva DNA Preservative was added to the saliva. The preserved saliva samples were then stored at 55°C for 6 months. Aliquots were removed and analyzed after 1 month, 2 months, 4 months and after 6 months. The saliva DNA was isolated from the saliva/preservative sample in duplicate using Norgen's Saliva DNA Isolation Kit (Cat# 45400) as per the recommended protocol.

Gel electrophoresis

For visual analysis 20 µL of DNA from the final DNA elution was loaded on to a 1% agarose TAE gel and run for 25 minutes at 150 V. The gel photo was taken using an AlphaImagerTM IS-2200 (Alpha Innotech).

PCR Amplification

The purified DNA was then used as the template in realtime PCR reactions. Briefly, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture (SYBR Green) containing 2.5 mM 5S primer pair. The PCR samples were amplified under the real-time program; 95°C for 5 minutes for an initial denaturation, 40 cycles of 95°C for 15 second for denaturation and 60°C for annealing and extension. The reaction was run on an iCycler iQ real-time system (Bio-Rad).

RESULTS AND DISCUSSION

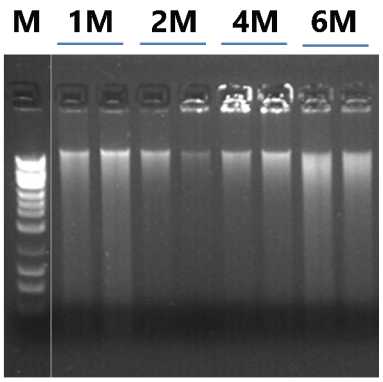
Saliva samples often need to be stored for a period of time prior to DNA isolation and analysis. Traditionally saliva samples are held on ice and then frozen at -20°C as soon as possible, however such storage leads to increased costsand is not always convenient, especially in resource-limited settings. The ability to safely store saliva samples at ambient temperatures without any degradation of the DNA is ideal. Norgen has developed a Saliva DNA Preservative which allows for the preservation of saliva samples at room temperature for more than 2 years prior to DNA isolation.

In addition to preservation at room temperature, an ideal preservative will also allow for preservation of saliva DNA at higher temperatures for extended periods of time. These higher temperatures may be encountered during shipping, when collected saliva samples are being shipped back to a laboratory for DNA extraction and further testing.

Also, elevated temperatures may be encountered when the saliva samples are collected in the field and are held prior to performing the DNA isolation and analysis. Therefore, in this application note, saliva samples are mixed with Norgen's Saliva DNA Preservative and stored at 55°C for up to 6 months, to determine the stability of the saliva DNA at elevated temperatures.

Saliva DNA was isolated from the preserved saliva samples after 1 month, 2 months, 4 months and 6 months of storage at 55°C. Aliquots of each elution were subsequently run on a 1% agarose TAE gel for visual analysis. As it can be seen in **Figure 1**, there is no change in DNA integrity after the saliva samples are stored for 6 months at 55°C in Norgen's Saliva DNA Preservative.

These DNA samples were then used as the template in a real-time PCR reaction to detect the 5S gene. DNA samplesmust be of a high quality in order to be used successfully in downstream amplification reactions. As it can be seen in **Figure 2**, the DNA isolated from the saliva samples stored at 55°C for up to 6 months could all be successfully amplified using real time PCR. Furthermore, the quality of the saliva DNA did not change from 1 month to 6 months, as indicated by the fact that the Ct value remained consistent. Therefore, saliva DNA can be successfully stored in Norgen's Saliva DNA Preservative 55°C for up to 1 year.



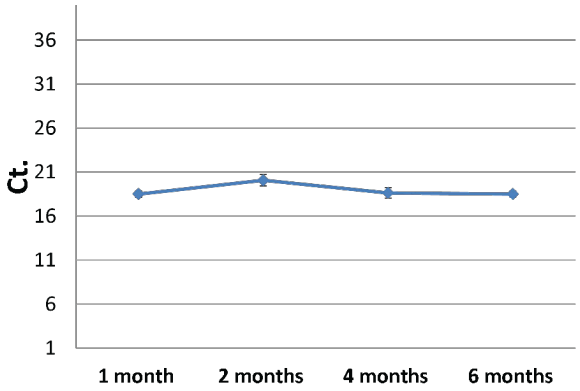


Figure 1. Stability of DNA preserved in Norgen's Saliva DNA Preservative at 55°C for up to 6 months. M: Norgen's UltraRanger 1 Kb DNA Ladder. (Cat#12100).

Figure 2. Graph of Ct values from real-time PCR reactions detecting the 5S gene from saliva DNA isolated from Norgen-preserved saliva stored at 55°C for up to 6 months. The quality of the DNA remained consistent over the 6 months.

CONCLUSIONS

High quality saliva DNA can be isolated and successfully amplified from saliva samples stored in Norgen's DNA Preservative at 55°C for up to 6 months.

Norgen is an ISO certified company, indicating our commitment to quality

Order Today!

Three ways to Order

Visit **norgenbiotek.com**
Call at **1-866-NORGENB** or **905-227-8848**
Email at **orders@norgenbiotek.com**

RELATED PRODUCTS

Description	Size	Cat. #
Saliva DNA Collection and Preservation Devices (50)	50 Devices	RU49000
Saliva DNA Isolation Kit	50 Prep	RU45400
UltraRanger 1 kb DNA Ladder	100 Loads	12100

For more information
SCAN HERE