

DNA Isolation from Saliva Preserved using Norgen's Saliva DNA Collection and Preservation Device using a Competitor's Advance DNA Kit

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INTRODUCTION

Saliva is a useful bodily fluid for diagnostic and research purposes. Collection is non-invasive and practical, as DNA isolated from saliva can be used for the screening and detection of biomarkers of cancer and autoimmune disorders, as well as for genotyping and more^{1,2}. Competitor 1's Advance DNA Kit is a popular kit for the purification of DNA from saliva for genotyping, as researchers have recently used it for genotyping the oxytocin receptor gene in mistreated children³, and the serotonin receptor in posttraumatic stress patients⁴.

Competitor 1's Advance DNA Kit is a paramagnetic bead system, built on reversible immobilization. It is useful for high-throughput studies, it is compatible with automated work stations, and it does not require centrifugation or vacuum manifold. In this study, we compared DNA isolation from Norgen-preserved saliva and competitor 2-preserved saliva using Competitor 1's Advance DNA Kit to determine the compatibility of both preservatives on this unique system.

MATERIALS AND METHODS

Sample collection

Saliva samples were collected from three different individuals. Four milliliters of saliva was collected from each participant. Half of each sample was preserved in Norgen's saliva preservative, while the other half was preserved using Competitor 2's preservative. A portion of these samples were pooled together to represent a general Norgen-preserved saliva sample, and an Competitor 2-preserved saliva sample. The rest of the samples were used to determine sample-to-sample variation among both preservatives.

Saliva DNA extraction

DNA was extracted from all saliva samples using the Advance DNA Kit, as per the manufacturer's protocol, with

saliva-specific modifications made to the binding procedure. Briefly, saliva (both Norgen and Competitor 2) was incubated at 55°C for 1 hour, prior to DNA isolation. For Norgen Preserved Saliva, Bind1 Buffer was not used, and instead replaced by 10 µL of Norgen's proteinase K, and the sample was incubated for 20 minutes at 55°C. After the incubation, 340 µL of Bind2 Buffer (containing the magnetic beads) was added to the tube. The lysate was mixed by pipetting, followed by incubation on the magnetic rack for 8 minutes. The subsequent wash and elution steps were followed as per the manufacturer's protocol. Saliva DNA was eluted in 50 µL of Elution Buffer. For Competitor 2-preserved saliva, the optimized protocol suggested by the manufacturer was followed. A Norgen-isolated control (spin column-based) was also used to compare yield and quality of Norgen-preserved saliva DNA isolated from the Norgen DNA Isolation Kit (Cat# 45400) and the Advance DNA kit, to confirm adaptability of the preservative to other systems.

Real-Time PCR

The purified DNA was then used as the template in a real-time PCR (qPCR) reaction. Briefly, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007) spiked with SYBR® Green dye, 2.5 mM 5S primer pair, and nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and 72°C for 45 seconds for extension. The reaction was run on an iCycler iQ realtime system (Bio-Rad).

RESULTS AND DISCUSSION

Saliva DNA was isolated from Norgen-preserved and Competitor 2-preserved saliva samples using the Advance DNA Kit, a magnetic bead system. A Norgen spin column control was also used (with DNA isolated using the manufacturer's protocol) as a positive control, as the Norgen saliva preservative has been optimized for this kit. A pooled saliva sample was used to assess overall compatibility of both preservatives using the

Advance DNA magnetic bead system. Fifteen microliters of 150 μ L of Norgen's spin column elutions, and 4 μ L of 40 μ L of the Advance DNA elutions were run on 1X TAE 1.0% agarose gel (**Figure 1**). It was found that the DNA isolated from the Norgen-preserved saliva samples on the Advance DNA system was even cleaner than the Norgen spin column control (which the preservative was optimized for), and the DNA yield was higher than the Competitor 2 preservative, which is the current leading market competitor. Next, to determine the quality of the DNA isolated using the Advance DNA magnetic bead system, 2 μ L of purified DNA were used in a 20 μ L qPCR reaction (SYBR Green®) using 5s rRNA primers (**Figure 2**). Based on the Ct values generated, the Norgen-preserved saliva and the Competitor 2-preserved saliva were of comparable quality, with both samples amplifying at a Ct of ~20. The Spin Column control was found to have a slightly lower Ct value, despite the Norgen saliva preservative being optimized for this kit. This demonstrates the compatibility of Norgen's saliva preservative with the Advance DNA magnetic bead system.

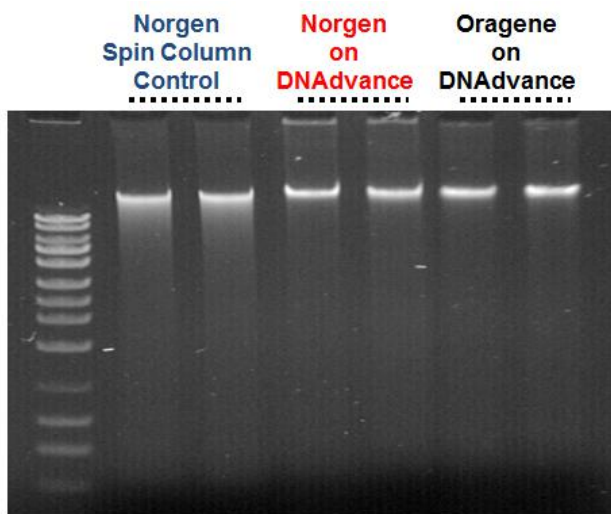


Figure 1. Comparison of DNA Yields from Norgen-Preserved and Competitor 2-Preserved Saliva on the Advance DNA System. Fifteen microlitres out of 150 μ L of Norgen's spin column elution, and 4 μ L out of 40 μ L of the Advance DNA elutions were loaded on a 1X TAE 1.0% gel. The Norgen-preserved saliva sample appears to generate higher DNA yields than Competitor 2-preserved saliva on the Advance DNA system, and the yield is also comparable to Norgen's spin column control.

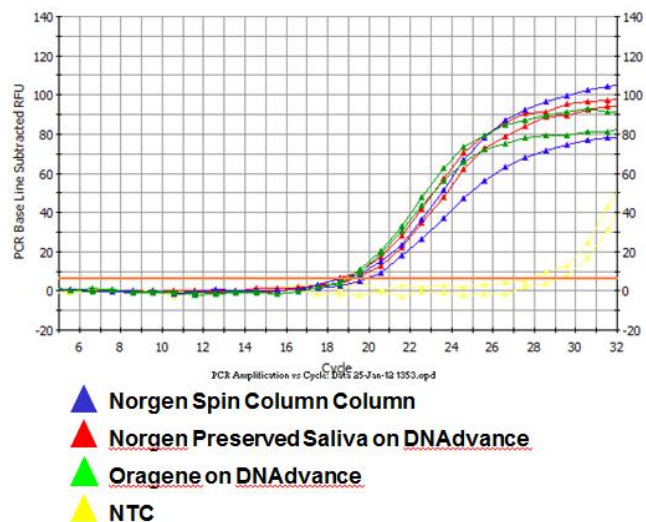


Figure 2. Comparison of qPCR Performance Using Norgen-Preserved and Competitor 2-Preserved Saliva DNA on the Advance DNA System. Two microliters of DNA eluted from the Advance DNA elutions and Norgen's spin column control were used in a qPCR reaction with primers flanking the 5s rRNA gene. The Norgen-preserved and Competitor 2-preserved saliva DNA performed similarly using Advance DNA, indicating that the Norgen preservative (while optimized for the Norgen Saliva DNA Isolation Kit) is compatible with a magnetic bead system, producing high quality DNA using both systems (Norgen spin column and Advance DNA). Both Norgen and Competitor 2 samples showed Ct values of ~20.

Individual saliva samples were also used to test both preservatives for sample-to-sample variations. Three individual saliva donors collected 2 mL of saliva, with half being preserved with Norgen's saliva preservative, and half being preserved with Competitor 2 saliva preservative. DNA was then isolated from all samples using the Advance DNA magnetic bead system. Four microliters (from 40 μ L elutions) of purified DNA was then run on a 1X TAE 1.0% gel (**Figure 3**). Norgen's preservative was found to generate higher DNA yields than the Competitor 2 preservative using the same saliva samples. This indicates that Norgen's preservative is more compatible with the Advance DNA magnetic bead system than our leading market competitor.

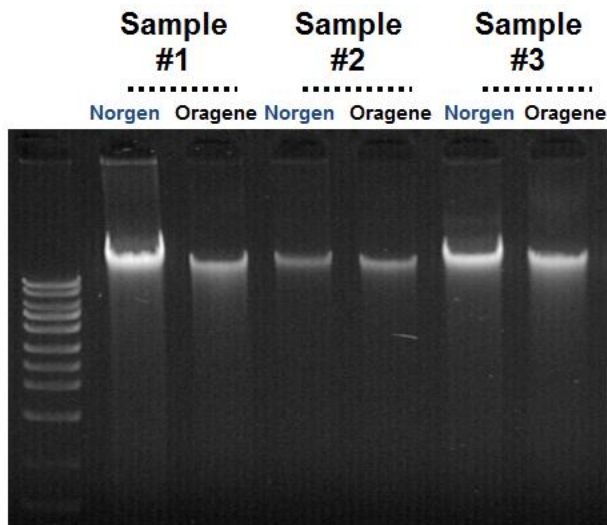


Figure 3. Comparison of DNA Yields from Three Different Saliva Donors Using Norgen's and Competitor 2 Saliva Preservative on the Advance DNA System. Four microliters out of 40 μ L of DNAAdvance elutions were loaded on a 1X TAE 1.0% gel. The Norgen-preserved saliva samples appear to generate higher DNA yields than Competitor 2-preserved saliva on the Advance DNA system, especially when the sample contains high amounts of DNA.

The saliva DNA purified from the samples in **Figure 3** were then quantified using the NanoVue™ spectrophotometer, as per the manufacturer's protocol. The yield from each is depicted in **Figure 4**. In correlation with the gel, Norgen-preserved saliva performed exceptionally well using the Advance DNA system, generating higher yields than the same samples preserved using Competitor 2 preservative.

Also generated from the NanoVue™ spectrophotometer readings, the A260:A280 and A260:A230 ratios were used to assess the quality of DNA isolated from both preservatives, using the Advance DNA system. The average and standard deviation generated from the three samples was then calculated for both ratios. The average A260:A280 ratio (**Figure 5**) was found to be very similar between the Norgen and Competitor 2-preserved saliva samples, however the average A260:A230 ratio (**Figure 6**) was found to be much higher for the Norgen-preserved saliva. These ratios are also very similar to the ratios generated from DNA isolated from Norgen-preserved saliva using the Norgen Saliva DNA Isolation Kit. These findings indicate that similar to Competitor 2, Norgen's saliva DNA preservative is compatible with a magnetic bead system, such as the Advance DNA Kit, offered by Competitor 2.

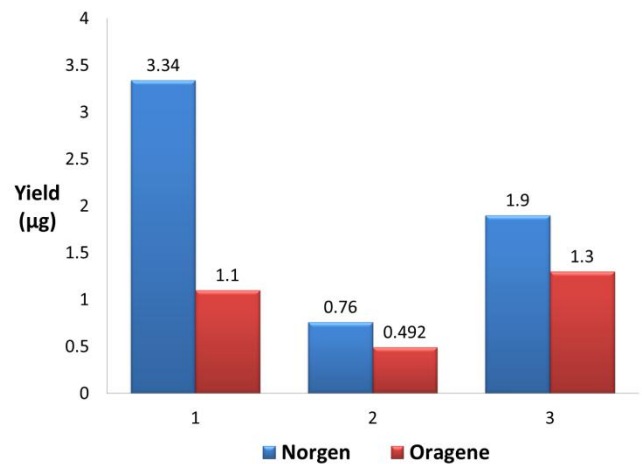


Figure 4. Comparison of DNA Yields from Three Different Saliva Donors Using Norgen's and Competitor 2 Saliva Preservative on the Advance DNA System. Saliva DNA samples were quantified using the NanoVue™ Plus spectrophotometer. The Norgen-preserved saliva samples generated higher DNA yields than Competitor 2-preserved saliva on the Advance DNA system for all three different saliva samples.

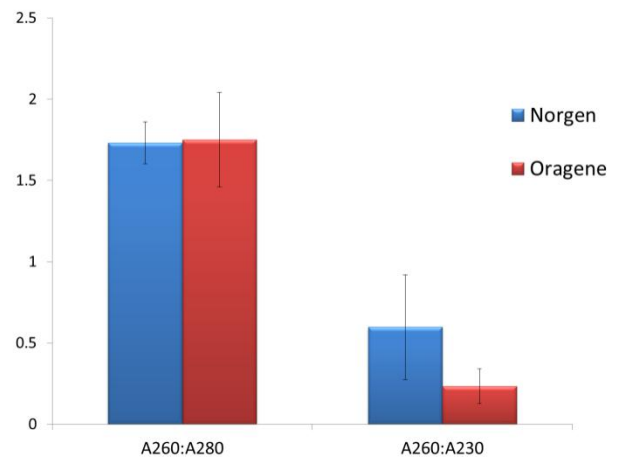


Figure 5. Comparison of DNA Quality from Norgen's and Competitor 2 Saliva Preservative on the Advance DNA System. Based on the three saliva samples used in Figures 3 and 4, the average A260:A280 and A260:A230 were calculated from reading given by the NanoVue™ Plus spectrophotometer. The Norgen and Competitor 2 preservatives gave similar A260:A280 ratios using the Advance DNA system, while the A260:A230 generated using the Norgen-preserved saliva samples was much higher (more than double) that of the Competitor 2-preserved saliva samples.

CONCLUSIONS

From the data presented in this report, the following can be concluded:

- 1. Norgen's Saliva DNA Preservative is Compatible with Magnetic Bead Systems.** Competitor 1's Advance DNA system was found to be fully compatible with Norgen's saliva DNA preservative, generating high quality DNA with comparable yields to the Norgen's Saliva DNA Isolation Kit (Cat# 45400).
- 2. Norgen's Saliva Preservative is Comparable to Competitor 2's Saliva Preservative on Magnetic Bead Systems.** Norgen's saliva preservative is as compatible if not more suited for magnetic bead systems as Competitor 2's preservative, based on yield, quality and qPCR Ct values.
- 3. Norgen's Saliva DNA Preservative Optimally Performs across a Variety of Saliva Samples.** Norgen's preservative produced higher quality and yields of DNA from different samples, compared to the leading competitor. Norgen's preservative outperformed Competitor 2's preservative on the Advance DNA system for both DNA yield across three different samples, as well as the average A260:A230. The average A260:A280 for both preservatives was similar.

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Related Products	Product #
Saliva DNA Collection, Preservation and Isolation Kit	RU35700
Saliva DNA Collection and Preservation Devices	RU49000
Saliva DNA Isolation Kit	RU45400

