

# Automation-friendly saliva DNA collection

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## Abstract

Saliva is a convenient alternative to blood as a biological sample in research and diagnostic applications because it can be collected non-invasively and with very limited training. DNAGard® Saliva HT is a new device designed for the efficient collection and automated processing of saliva DNA for genetic analyses. The device has several features that make it useful for saliva collection and automated sample processing. These include an integrated stabilizer and spill-proof design that minimizes donor exposure to chemicals and contamination, and increases donor compliance. Moreover, a pierce-able cap eliminates the need to decap the device prior to laboratory processing of samples. Lastly, triple redundant, bar-coded labels, further support automated processing and sample analysis. The integrated stabilizer in DNAGard® Saliva HT contains optimized chemistry that provides preservation of DNA upon saliva collection. The DNAGard® Saliva HT stabilizer provides high yields of high purity DNA from human saliva stored for at least 10 months at 22°C, based on accelerated stability testing. Furthermore, DNA isolated from saliva samples stored in DNAGard® Saliva HT has high performance in long-range PCR and qPCR applications.

## Workflow Overview



## Materials and Methods

### Saliva Collection and Storage:

1. Saliva samples from five (5) donors were collected into conical tubes. Samples from each individual donor were mixed before allocating into DNAGard® Saliva HT solution (DGS-HT) or competitor O's solution (CO) at a 1:1 (vol:vol) ratio in duplicates. In addition, equal volume of saliva samples were stored alone as the non-protected control (NP). All samples were stored at 45°C for five (5) weeks and moved to 60°C for an additional nine (9) days, equivalent to 10 months at 22°C.
2. A second set of saliva samples from three donors with low, medium, and high DNA yield was collected and allocated under the same conditions, but stored at room temperature for three (3) weeks.

### Automated DNA Purification:

1. 500 µL aliquots of each sample from the first set of donors were removed and processed on a MagNA Pure Compact Instrument using MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume (Roche, Cat. No. 03730972001) according to the manufacturer's instructions.
2. 600 µL aliquots of each sample from the second set of donors were removed, incubated at 56°C overnight, and processed on a chemagic Magnetic Separation Module I Instrument using chemagic DNA Saliva Kit (PerkinElmer chemagen, Cat. No. CMG-1081) according to the manufacturer's instructions.

**DNA Yield and Purity:** Total DNA yield and purity were quantified by UV spectrophotometry. In addition, dsDNA was quantified using a Quant-iT™ PicoGreen® dsDNA Assay kit (Thermo Fisher, Cat. No. P7589). Approximately 5% of purified DNA from each sample was analyzed by agarose gel electrophoresis and stained with ethidium bromide.

**Long-range PCR:** DNA integrity was analyzed by long-range PCR amplification of a 3.9 kb fragment of the human GAPDH gene using KOD Xtreme™ Hot Start PCR kit (Novagen) on the iCycler Thermal Cycler (Bio-Rad), followed by electrophoresis and stained with ethidium bromide.

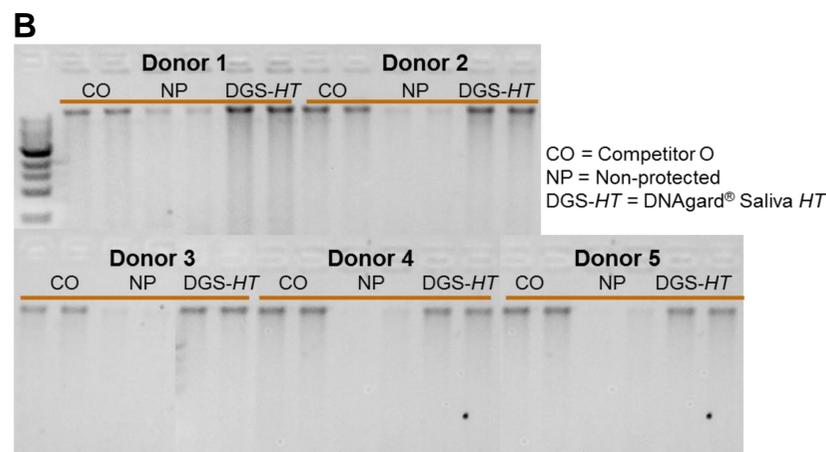
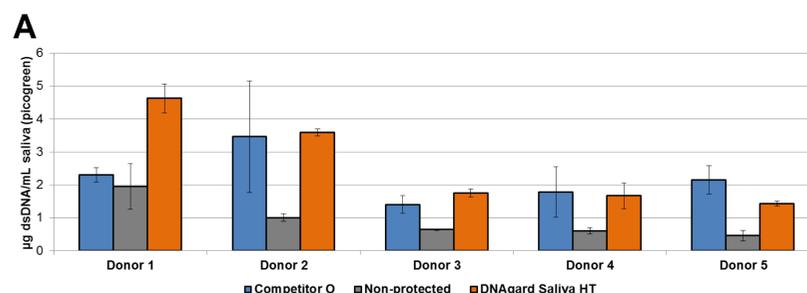
**qPCR:** Approximately 1% of the purified DNA from each sample was quantified by qPCR amplification of an RNaseP amplicon, using iQ™ SYBR® Green Supermix (Bio-Rad, Cat. No. 170-8880) on a CFX96 Real-Time PCR Instrument (Bio-Rad).

## Results

### DNA Isolation using the automated systems

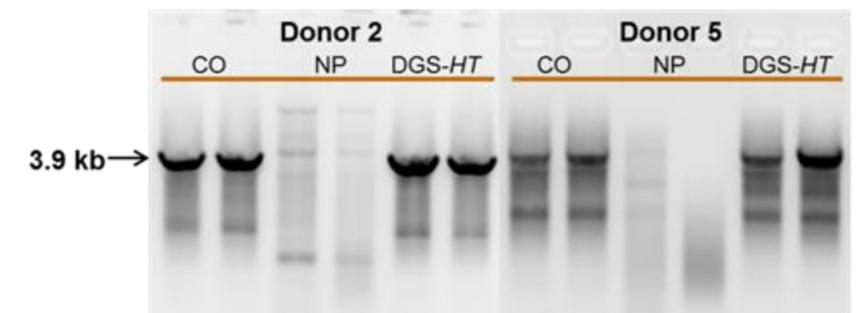
|               | Total dsDNA/ml saliva (µg) | Total NA/ml saliva (µg) | A <sub>260</sub> /A <sub>280</sub> ratio | Purification Kit   | Platform                              |
|---------------|----------------------------|-------------------------|--|--|---------------------------------------|
| DGS-HT        | 2.56 ± 1.33                | 11.54 ± 9.47            | 2.16 ± 0.88                              | MagNA Pure Compact Nucleic Acid Isolation Kit I - Large Volume | MagNA Pure Compact System             |
| Non-protected | 0.91 ± 0.60                | 4.93 ± 2.34             | 2.20 ± 0.44                              |  |                                       |
| Competitor O  | 2.18 ± 0.96                | 5.07 ± 3.34             | 1.73 ± 0.71                              |  |                                       |
| DGS-HT        | 4.36 ± 3.08                | 12.38 ± 8.15            | 1.80 ± 0.27                              | chemagic DNA Saliva Kit  | chemagic Magnetic Separation Module I |

**Table 1: Summary of results of pure salivary DNA from duplicate samples per donor.** DNA isolated on the MagNA Pure System was from duplicate saliva samples from five (5) donors after 44 days of storage under high temperature conditions (80% at 45°C, followed by 20% at 60°C). DNA isolated with chemagic DNA Saliva Kit was from duplicate saliva samples from three (3) donors after three (3) weeks of storage. dsDNA was quantified using a Quant-iT™ PicoGreen® dsDNA Assay kit. Total Nucleic Acid yield and purity (A<sub>260</sub>/A<sub>280</sub>) was analyzed by UV spectrophotometry.

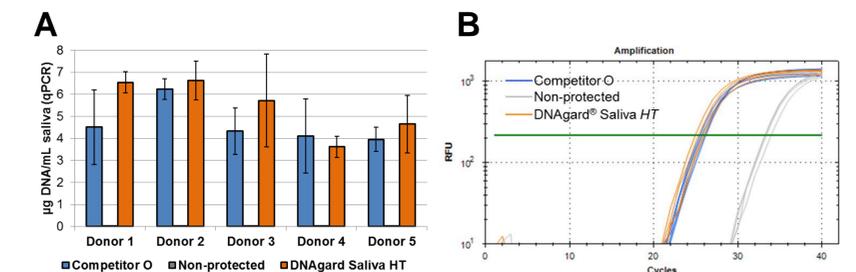


**Figure 1: High integrity and yield of DNA isolated by the MagNA Pure System from saliva stored in DNAGard® Saliva HT solution.** Duplicate saliva samples per donor from five donors were analyzed at Day 44 after 5 weeks at 45°C and 9 days at 60°C storage. dsDNA yield was measured by Quant-iT™ PicoGreen® dsDNA Assay (A) and DNA integrity was assessed by agarose gel electrophoresis (B).

### Salivary DNA performance in downstream applications: long-range PCR & qPCR



**Figure 2: Assessment of DNA integrity by long-range PCR.** DNA integrity from the 44-day old DNAGard® Saliva HT samples (DGS-HT) was assessed by long-range PCR amplification of a 3.9 kbp region of the GAPDH gene and compared to amplification from Competitor O-stabilized DNA (CO) and non-protected DNA (NP).



**Figure 3: Genomic DNA content in saliva samples isolated after 44 days of storage under high temperature conditions.** The DNA purified from DNAGard® Saliva HT samples, Competitor O-stabilized DNA, and non-protected DNA was quantified by qPCR amplification of an RNaseP target as a function of input nucleic acid. Average DNA per milliliter of saliva from duplicate samples per donor (A) and example of amplification curve from donor 3 (B) are shown.

## Summary

Automated saliva DNA purification using DNAGard® Saliva HT following saliva storage at elevated temperatures provides the following results:

- DNA is stabilized in human saliva for at least 10 months at 22°C, based on accelerate aging.
- High quality DNA is isolated from DNAGard® Saliva HT using the MagNA Pure System (Roche) and chemagic Magnetic Separation Module I Instrument (PerkinElmer chemagen):
  - High purity DNA (Table 1)
  - DNA yield > 2.56 µg/ mL saliva (Figure 1)
  - Intact high molecular weight DNA (Figure 2)
- High performance in long-range PCR and qPCR applications of DNA purified from DNAGard® Saliva HT (Figures 2 and 3)