

## DNA purification from saliva stabilized in DNAgard® Saliva using QIAamp® Blood Mini Kit

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### Introduction

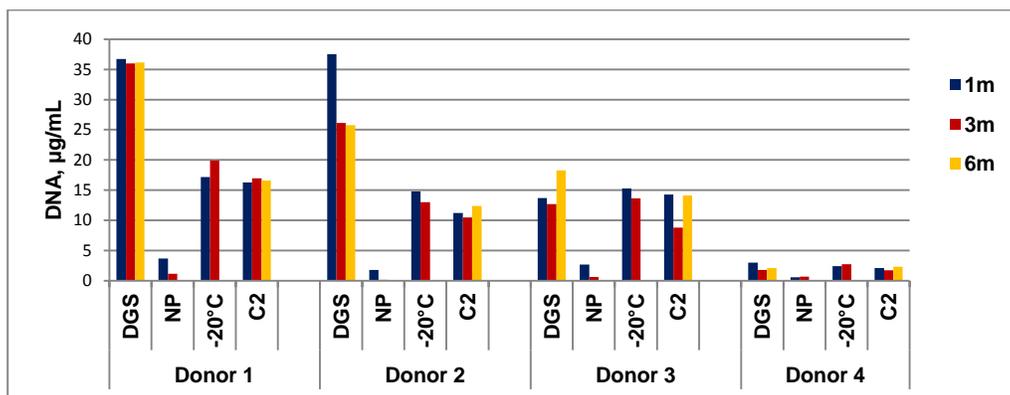
DNAgard® Saliva (Cat# 97021-011A) allows for the efficient collection, preservation, shipping and storage of saliva samples for DNA purification and analysis. The product is designed for ease of use, with a simple process from collection to storage. DNA in saliva samples are preserved for up to 2 years at room temperature, with high quality DNA recovery. The stabilization offers flexible DNA recovery from a wide range of DNA purification kits. In this study, we show DNA purification from human saliva samples stabilized in DNAgard Saliva using QIAamp® Blood DNA Extraction Mini Kit (Cat# 51104, QIAGEN).

### Materials and Methods

**Saliva sample processing and DNA extraction:** Saliva was collected from four donors. 2 mL of saliva was mixed with DNAgard Saliva (1.5 mL) and a competitor's saliva stabilizer (2 mL) according to the manufacturers' protocols. The saliva samples were stored at room temperature for 1, 3 and 6 months prior to DNA extraction. DNA was extracted from each sample using QIAamp® Blood DNA Extraction Mini Kit. Below is the DNA purification protocol for 350 µL aliquot of DNAgard Saliva samples.

1. Add 30 µL Protease (or Proteinase K) to the 350 µL sample. Mix gently.
2. Add 300 µL Buffer AL to the sample. Vortex for 15 seconds.
3. Incubate at 56°C for 10 minutes.
4. Briefly centrifuge the tubes to remove drops from the inside of the lid.
5. Add 300 µL ethanol (100%) to the sample. Vortex for 15 seconds and then centrifuge briefly to remove drops from the inside of the lid.
6. Carefully apply the mixture to the QIAamp mini spin column (in a 2 mL collection tube) without wetting the rim. Close the cap and centrifuge at 8000 rpm for 1 minute. Place the column in a clean 2 mL collection tube and discard the tube containing the filtrate.
7. Add 500 µL Buffer AW1 to the spin column. Centrifuge at 8000 rpm for 1 minute. Discard the flow-through and place the column in the collection tube.
8. Carefully open the QIAamp mini spin column and add 500 µL buffer AW2. Centrifuge at full speed for 3 minutes.
9. Place the column in a new 2 mL collection tube and centrifuge at full speed for 1 minute.
10. Place the column in a clean 1.5 mL microcentrifuge tube. Add 100 µL Buffer AE or distilled water. Incubate at room temperature for 1 minute, and then centrifuge at 8000 rpm for 1 minute.

**DNA analysis:** DNA from each sample was quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit (Cat# P11496, Invitrogen).



**Figure 1.** DNA quantity from saliva stabilized with DNAgard Saliva and other methods. The saliva samples stabilized with DNAgard Saliva (DGS) or a competitor's stabilizer (C2) were stored at room temperature for 1, 3, and 6 months as indicated. Saliva without any stabilizer from each donor was stored at room temperature for the same period of time as a negative control (NP). A positive control (-20°C) is saliva from each donor stored at -20 °C. DNA was extracted as described above. DNA from each sample was quantified using Quant-iT™ PicoGreen® dsDNA Assay Kit. All samples were extracted and analyzed in duplicates.

## Results and Summary

In this study, we compared DNA quantity obtained from saliva samples stabilized by DNAGard Saliva with other stabilizers. Our result has demonstrated that the DNA quantity from saliva stabilized with DNAGard Saliva remained stable over a six month period. Over two folds of DNA recovery were observed in the saliva samples preserved by DNAGard Saliva after a month of storage at room temperature compared to the saliva stored at -20°C or with the competitor's stabilizer (Donors 1 & 2). In summary, DNAGard Saliva showed high DNA recovery in human saliva samples. It provides an efficient way for collection and transport of saliva specimens at room temperature.

**Note:** Please read all instructions for the [DNAGard Saliva](#) prior to using this protocol.

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