

DNA Purification from Saliva Stabilized in DNAGard® Saliva Using Manual and Automated DNA Purification Methods

I. Saliva sample collection

1. The subjects must rinse their mouths with water to remove food at least 30 minutes prior to donating saliva and should not eat or drink in the intervening time between mouth rinsing and sample collection.
2. Deposit 2 mL of saliva into the collection device. Remove the collection funnel, and add the DNAGard Saliva reagent to the saliva sample in the tube. Cap the tube, and quickly mix by inversion 5-7 times. Please refer to [DNAGard Saliva Instruction for Use](#) for detailed procedures. The saliva mixture is now ready to ship or store.

II. Saliva sample treatment prior to DNA purification

To begin the extraction of saliva DNA, vortex the saliva sample at speeds sufficient to disperse all the saliva components for approximately 15 seconds or until no precipitation is visible.

III. Purify the saliva DNA with one of the following DNA purification methods

- [QIAamp® Blood DNA Mini Kit \(Cat. No. 51104, QIAGEN\)](#). See A below.
- [MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume \(Cat. No. 03 730 972 001, Roche Applied Science\)](#). See B below.
- [Gentra® Puregene® Blood Kit \(Cat. No. 158467, QIAGEN\)](#). See C below.
- Organic extraction method. See D below.

A. Purify saliva DNA using QIAamp® Blood DNA Mini Kit according to the following protocol. Please refer to [QIAamp DNA Blood Mini Kit](#) for more information.

1. Pipet 20 µL of QIAGEN protease (or proteinase K) to a 1.5 mL microcentrifuge tube.
2. Add 200 µL of saliva/stabilizer mixture to the tube.
3. Add 200 µL of Buffer AL to the tube. Vortex for 15 seconds.
4. Incubate the tube at 56°C for 10 minutes with shaking at 1000 rpm. If a shaker is not available, vortex the tube for 15 seconds after incubation.
5. Briefly centrifuge the tubes to remove drops from the inside of the lid.
6. Add 200 µL of ethanol (96-100%) to the tube. Vortex for 15 seconds, and then briefly centrifuge the tube to remove drops from the inside of the lid.
7. 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 new 2 mL collection tube, and discard the tube containing the filtrate.

8. Add 500 µL of Buffer AW1 to the spin column. Centrifuge at 8000 rpm for 1 minute. Place the column in a new collection tube, and discard the tube containing the filtrate.
9. Add 500 µL of buffer AW2 to the spin column. Centrifuge at full speed (14000 rpm) for 3 minutes.
10. Place the spin column in a new 2 mL collection tube, and discard the old collection tube with the filtrate. Centrifuge at full speed for 1 minute.
11. Place the spin column in a clean 1.5 mL microcentrifuge tube, and discard the collection tube containing the filtrate. Add 100 µL of Buffer AE or distilled water to the column. Incubate at room temperature for 1 minute, and then centrifuge at 8000 rpm for 1 minute.
12. Add additional 100 µL of Buffer AE or distilled water to the column. Incubate at room temperature for 1 minute, and then centrifuge at 8000 rpm for 1 minute.
13. Collect the total 200 µL of elution for DNA Analysis.

B. Purify saliva DNA using MagNA Pure Compact RNA Isolation Kit I-Large Volume with [MagNA Pure Compact Instrument](#) (Cat No 03731146001, Roche Applied Science)

1. Add 1 mL of saliva/stabilizer mixture to a sample tube provided in the MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume.
2. Place the sample tube in the MagNA Pure Compact tube rack, and start the “DNA_Blood_1000” protocol as described in the manual of the MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume.

C. Purify saliva DNA using Gentra® Puregene® Blood Kit

Please refer to the Application Note [DNA purification from saliva stabilized in DNAgard® Saliva using the Gentra® Puregene® Kit](#) in the *Resources* section of <http://www.biomatrica.com/dnagardsaliva.php>.

D. Purify saliva DNA using an organic extraction method

Please refer to the Application Note [DNA purification from saliva stabilized in DNAgard® Saliva using an organic extraction method](#) in the *Resources* section of <http://www.biomatrica.com/dnagardsaliva.php>.

- IV. Quantify DNA using [Quant-iT™ PicoGreen® dsDNA Assay Kit](#) (Cat# P11496, Life Technologies) or other methods. For more information, please refer to the manual of the [Quant-iT™ PicoGreen® dsDNA Assay Kit](#).