DNAgard® Saliva *HT* User Manual

For room temperature collection and preservation of DNA in saliva samples

For Research Use Only. Not for use in diagnostic procedures.

Biomātrica®

DNAgard® Saliva *HT*

Instructions for collecting, storing, and processing saliva samples for DNA isolation

Cat. No: 97021-012A (DNAgard® Saliva *HT*: 48 kits)

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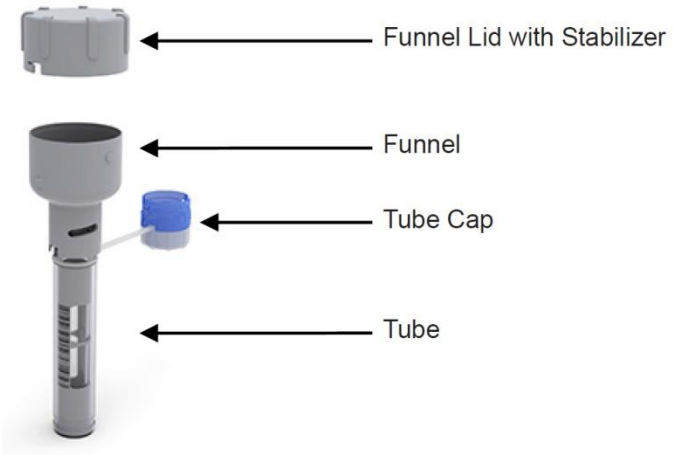
Product Description

DNAgard® Saliva *HT* is designed for efficient collection, preservation, shipping, storage, and automated processing of human saliva samples for DNA purification and analysis. DNAgard® Saliva *HT* prevents degradation of DNA found in saliva, enabling a simple, non-invasive method for collecting, storing, and transporting samples. Saliva DNA samples collected using DNAgard® Saliva *HT* are stable at room temperature (15°C to 30°C) for at least 12 months, and yield high quality DNA using a wide range of DNA purification kits.

Contents and Storage

Catalog No. 97021-012A consists of 48 DNAgard® Saliva *HT* tube kits. Each kit consists of the following items:

Item	Quantity
Package insert	One (1) per kit
Funnel Lid with stabilizer	One (1) per kit
Collection device Funnel Tube Cap Tube	One (1) per kit



DNAgard® Saliva *HT* kits are shipped and stored at ambient room temperature (15°C to 30°C) until the expiration date printed on the product. Avoid direct exposure to sunlight.

Safety and Warnings ⚠

This product is intended solely for the safe collection of saliva samples. Do NOT ingest the DNAgard® Saliva *HT* solution. Wear laboratory gloves, lab coat, and protective eyewear when handling this product. If solution comes in contact with eyes or skin, wash affected areas thoroughly with water. In case of accidental ingestion or contact with skin, refer to the SDS (formerly known as MSDS) available at www.biomatrica.com.

The contents of the DNAgard® Saliva *HT* solution may cause irritation to eyes, respiratory system, and skin.

1. If accidental inhalation occurs, supply fresh air and seek medical advice when necessary.
2. If skin contact occurs, wash immediately with soap and water, and rinse thoroughly.
3. If eye contact occurs, rinse immediately with plenty of water for at least 15 minutes and seek medical advice.
4. If accidental ingestion occurs, seek medical advice immediately.

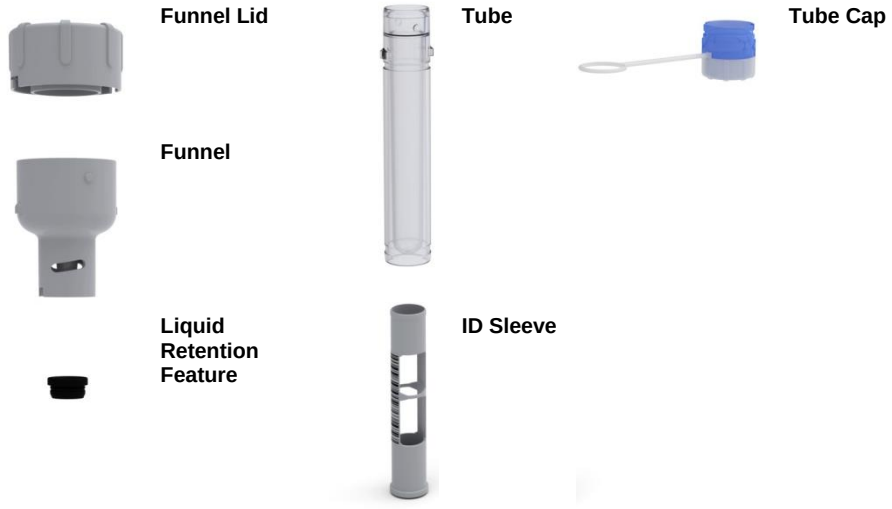
Introduction

Principle of Procedure

The DNAgard® Saliva *HT* kit is used to collect, preserve, store, and ship human saliva samples for DNA purification and analysis. The funnel lid contains 2 mL of DNAgard® stabilization solution. Each tube requires 1.7 mL of saliva for a total mixture volume of 3.7 mL. High quality DNA can be isolated from the preserved saliva using a wide range of commercially available DNA purification kits.

Product Features

As a biological sample, saliva is a convenient alternative to blood because it can be collected non-invasively and with very limited training. DNAgard® Saliva *HT* is designed for the efficient collection and automated processing of saliva DNA for genetic analyses. The collection device has several features that make it useful for saliva collection and automated sample processing, including an integrated stabilizer in the funnel lid and a liquid retention feature that minimizes donor exposure to chemicals and contamination. The integrated stabilizer is optimized to immediately preserve DNA upon saliva collection. The pierce-able tube cap eliminates the need to uncap the device prior to laboratory processing of samples. Lastly, laser-etched, triple redundant identification markers (1D, 2D, and alphanumeric barcoded labels) on the ID sleeve support automated processing and sample analysis. DNA isolation and purification are achieved using commercially available kits with clinical laboratory validated methods. High yields of high quality DNA can be used in a wide range of downstream research applications, including PCR and NGS-based assays.



Specimen Collection

⚠ Collection Precautions

1. Do NOT eat, drink, smoke, or chew gum 30 minutes prior to providing saliva sample.
2. Rinse mouth with water 30 minutes prior to providing saliva sample.
3. Store DNAgard® Saliva *HT* kits at room temperature (15°C to 30°C) prior to saliva collection.
4. If stabilizing solution comes in contact with eyes or skin, thoroughly wash with water.
5. Do not ingest stabilizing solution.
6. The tethered tube cap can be a potential choking hazard.

Procedure for Specimen Collection

1. Spit into the saliva collection tube until saliva (excluding bubbles) reaches the fill line marked on the tube.
   Note: This may take a few minutes. Saliva production can be stimulated by gently rubbing tongue on roof of mouth or by sucking on the inside of cheeks.
2. Holding the tube upright, screw the funnel lid onto the funnel, ensuring it is securely fastened. Solution is dispensed from the funnel lid into the tube and mixed with the saliva. *Note: Be careful not to spill any solution.*
3. Wait 10 seconds to ensure that all of the stabilizing solution has flowed into the tube. Unscrew and discard the funnel and funnel lid.
4. Screw the tethered tube cap securely onto the saliva collection tube, ensuring there is no leakage.
5. Mix by shaking vigorously for ten (10) seconds. The stabilized saliva mixture is now ready to ship, store, or process for DNA purification.

DNA purification: DNA from saliva stored in DNAgard® Saliva *HT* tubes can be purified using commercially available kits. Validated DNA purification methods are included in this manual on page 12.

Manual Sample Removal Recommendations

While DNAgard® Saliva *HT* tubes are designed for use with liquid handling robots, it is still possible to use them when such equipment is not available. Use the following procedure to efficiently remove samples with a manual pipette and disposable pipette tips (or similar method).

1. Wear proper laboratory protection (glasses, gloves, lab coat, etc.).
2. Mix each sample by shaking vigorously for 10 seconds or by briefly vortexing.
   Note – For easier handling, samples may be heated at 55 °C for 30 minutes prior to mixing. Mixed samples will remain homogenous for several hours after mixing. Samples stored longer than 12 hours should be remixed to ensure homogeneity.
3. Briefly centrifuge tubes to ensure any liquid trapped above the liquid retention feature flows back into the tubes.
4. Unscrew the tube cap from each tube.
5. Remove the liquid retention feature by gently prying it out of each tube using a disposable pipette tip or similar object.
6. Remove the sample from each tube.
   Note – Samples should be removed from the midpoint between the meniscus and the bottom of each tube.

   Precaution – Removing samples through the pierce-able septa using a manual pipette is NOT recommended because friction between the septa seal and the liquid retention feature can cause the tip to be dislodged from the pipette.
7. Close each tube with the tube cap.

Automated Sample Removal Recommendations

The following guidelines are suggested when using DNAgard® Saliva *HT* tubes with an automated liquid handling robot. Refer to the manufacturer's instructions for proper robot use. Contact the manufacturer for robot accessories such as racks and lock down modules.

Sample Removal with a Fixed Probe

1. Wear proper laboratory protection (glasses, gloves, lab coat, etc.).
2. Mix the samples by shaking vigorously for 10 seconds or by briefly vortexing.
   Note - Mixed samples will remain homogenous for several hours following mixing. Samples stored longer than 12 hours should be remixed to ensure homogeneity. Samples can also be mixed by vigorously pipetting at least 1 mL sample volume from each tube during sample removal (step 7).
3. Load samples into a carrier rack.
4. Place the carrier rack in the robot and affix the lock down module.
   Note – Depending on the make and model of the instrument, the lock down module may be built into the carrier rack. Contact your instrument manufacturer for assistance.
5. Program the X-Y position for the center of the tube septa.
6. Program the desired sampling depth.
7. Set the instrument to homogenize each sample by mixing vigorously, and to remove the desired volume from each tube.
   Note – Samples should be removed from the midpoint between the meniscus and the bottom of the tube.
8. Move each sample to a processing plate.
9. Rinse the tip between samples.
10. Repeat steps 7 through 9 until all samples have been transferred.

Sample Removal with Disposable Tips

When using disposable tips, Biomatrica recommends using narrow pipette tips and a robot capable of ensuring that tubes remain on the pipette head (e.g., the Hamilton Company CO-RE® system) at all times.
Note – Friction generated from the tube cap septa and liquid retention feature can dislodge a large pipette tip from the pipette head of some robots.

1. Wear proper laboratory protection (glasses, gloves, lab coat, etc.).
2. Mix the samples by shaking vigorously for 10 seconds or by briefly vortexing.
   Note - Mixed samples will remain homogenous for several hours following mixing. Samples stored longer than 12 hours should be remixed to ensure homogeneity. Samples can also be mixed by vigorously pipetting at least 1 mL sample volume from each tube during sample removal (step 8).
3. Load samples into a carrier rack.
4. Place carrier rack in the robot and affix the lock down module.

Note – Depending on the make and model of the instrument, the lock down module may be built into the carrier rack. *Contact the instrument manufacturer for assistance.*

5. Program the X-Y position for the center of the tube septa.
6. Program the desired sampling depth.
7. Load tip(s) onto the pipette head.
8. Set the instrument to homogenize each sample by mixing vigorously, and to remove the desired volume from each tube.
   Note – Samples should be removed from the midpoint between the meniscus and the bottom of each tube.
9. Move each sample to a processing plate.
10. Dispose of the pipette tip(s).
11. Repeat steps 7 through 10 until all samples have been transferred.

Automated DNA Purification General Recommendations

The following procedure is provided as a general guideline for successful use of DNAgard® Saliva *HT* tubes. Further optimization may be required as individual laboratory conditions can vary.

Note – Preheating the DNAgard® Saliva HT stabilizer mixture prior to sample purification is NOT necessary as it does not increase yields compared to unheated samples.

1. The DNAgard® Saliva *HT* stabilizer is an ionic cell lysing solution. Biomatrica recommends replacing the lysis buffer provided in many kits with water as salts in the lysis buffer may interfere with DNA binding to the beads.
2. Saliva-stabilizer mixes are often highly viscous, requiring additional mixing of the capture beads during the binding step.
    a. For instruments that use pipettes, Biomatrica recommends pipetting at a minimum speed of 500 µl per second, and NOT engaging the delay settings.
    b. For instruments that use plunger mixing (e.g. Thermo Fisher KingFisher™, Perkin Elmer MSM1, Qiagen QiaSymphony®), Biomatrica recommends mixing the binding beads by alternating between fast and medium speeds over a 10 minute period. Downstream washing with alcohol solutions should be carried out using the same settings. The final purification steps should include a wash in 80% ethanol/20% nuclease free water, a 6-10 minute air drying step in place of water rinses, and an elution carried out at 72°C with slow to medium mixing.

DNA Purification from DNAgard® Saliva *HT* Tubes

Saliva samples stabilized using DNAgard® Saliva *HT* are compatible with a wide range of purification chemistries. Biomatrica has tested several chemistries to ensure compatibility. The table below provides chemistry- and instrument-specific recommendations based on internal testing. Additional manual methods, including spin columns, organic extraction, and ethanol extraction of DNA have been successfully tested with DNA collected using DNAgard® Saliva *HT* tubes.

Purification Chemistry	Purification Instrument	Recommended Sample Volume	Additional Recommendations
PerkinElmer chemagic DNA Blood Kit special	PerkinElmer MSM1	1 mL	• Mix during binding as described above in 2b
Qiagen QiaSymphony® DSP DNA Kit	Qiagen QiaSymphony®	400 µL	• Use protocol Blood 400 • Replace lysis buffer with water • Mix during binding as described above in 2b
Machrey Nagel NucleoMag® Blood 200 µL Kit	Thermo Fisher KingFisher™	400 µL	• Use protocol outlined in manual • Replace lysis buffer with water • Mix during binding as described above in 2b
Roche MagNA Pure Compact Nucleic Acid Isolation Kit I-Large Volume	Roche MagNA Pure	1 mL	• Use protocol DNA_Blood_1000
Beckman Coulter Agencourt® DNAdvance® DNA Isolation Kit	Beckman Coulter Biomek® FXP	250 µL	• Use protocol in DNAdvance® Reagent Kit manual • Replace lysis buffer with water • Mix during binding as described above in 2a

DNA Quantification Recommendations

Fluorescence Method

Compared to traditional methods using absorbance at 260 nm, assays that use fluorescent dyes can precisely detect double-stranded DNA (dsDNA) in the presence of ssDNA, RNA, and free nucleotides. Biomatrica recommends using the fluorescent dyes in commercially available kits such as the Thermo Fisher's Quant-iT™ PicoGreen dsDNA Assay Kit (Cat. No. Q-33130) to quantify DNA purified from DNAgard® Saliva *HT* tubes because there is less interference from contaminating RNA. Biomatrica further recommends diluting the purified DNA to 1:50 with TE solution and using 5 μL in the quantification assay.

Absorbance Method

When quantifying DNA by absorbance, Biomatrica recommends first treating purified samples with RNase to digest contaminating RNA, and removing the RNA fragments by ethanol precipitation of the DNA.
Note – DNA from an oral sample typically contains appreciably more RNA than found in blood samples. Ensure that alcohol-precipitated DNA is fully dissolved before reading the absorbance.

Conversion factor: For pure dsDNA, an absorbance of 1.0 at 260 nm corresponds to a concentration of 50 ng/μL (50 μg/mL).

Method:

1. Mix purified RNase-treated DNA by vortexing or pipetting up and down. Wait for bubbles to clear.
   Note – Cloudy purified DNA samples (usually resulting from magnetic bead purification) should be centrifuged briefly before taking an absorbance reading.
2. Use the appropriate elution buffer in the reference (blank) cell.
3. Measure absorbance at 320 nm, 280 nm and 260 nm with corresponding corrected path length.
4. Calculate corrected A_{280} and A_{260} values by subtracting the absorbance at 320 nm (A_{320}) from the A_{280} and A_{260} values.
   Corrected $A_{280} = A_{280} - A_{320}$
   Corrected $A_{260} = A_{260} - A_{320}$
5. DNA concentration in ng/μL = corrected A_{260} x (dilution factor) x 50 (conversion factor).
   For undiluted DNA sample: [DNA] ng/μL = corrected A_{260} x 1 x 50
6. A_{260}/A_{280} ratio: Divide corrected A_{260} by corrected A_{280}.

Example:
Assume the measured $A_{320} = 0.178$, $A_{280} = 0.399$ and $A_{260} = 0.583$.

The DNA concentration of the undiluted sample is: (A_{260} - A_{320}) x dilution factor x conversion factor.

= (A_{260} - A_{320}) x 1 x 50
= (0.583 - 0.178) x 1 x 50
= 0.405 x 1 x 50
= 20.25 ng/µL or 20.25 µg/mL

The corrected A_{260}/A_{280} ratio is: (A_{260} - A_{320}) ÷ (A_{280} - A_{320})

= (0.583 - 0.178) ÷ (0.399 - 0.178)
= 0.405 ÷ 0.221
= 1.83

Technical Assistance

Biomatrica, Inc. takes pride in providing efficient, quality technical support. Biomatrica's Technical Service Department is staffed by experienced scientists with extensive expertise in molecular biology and the use of Biomatrica's biostability and storage products. Please contact Biomatrica directly with any questions regarding DNAgard® Saliva *HT* technology, product use, or general matters.

Technical Service Department
Phone (US): (866) DRY-MTRX or (866) 379-6879
Web: www.biomatrica.com
Email: info@biomatrica.com

Label Information

	Catalog number		LOT number: Batch number
	Expiry Date. Collect saliva by the end of the month indicated.		Manufacturer
	Consult package insert		Caution, consult instructions for use
	Storage instructions		

DNAgard® is a registered trademark of Biomatrica.

Manufactured for: Biomatrica Inc.
5627 Oberlin Drive, #120
San Diego, CA 92121, U.S.A.