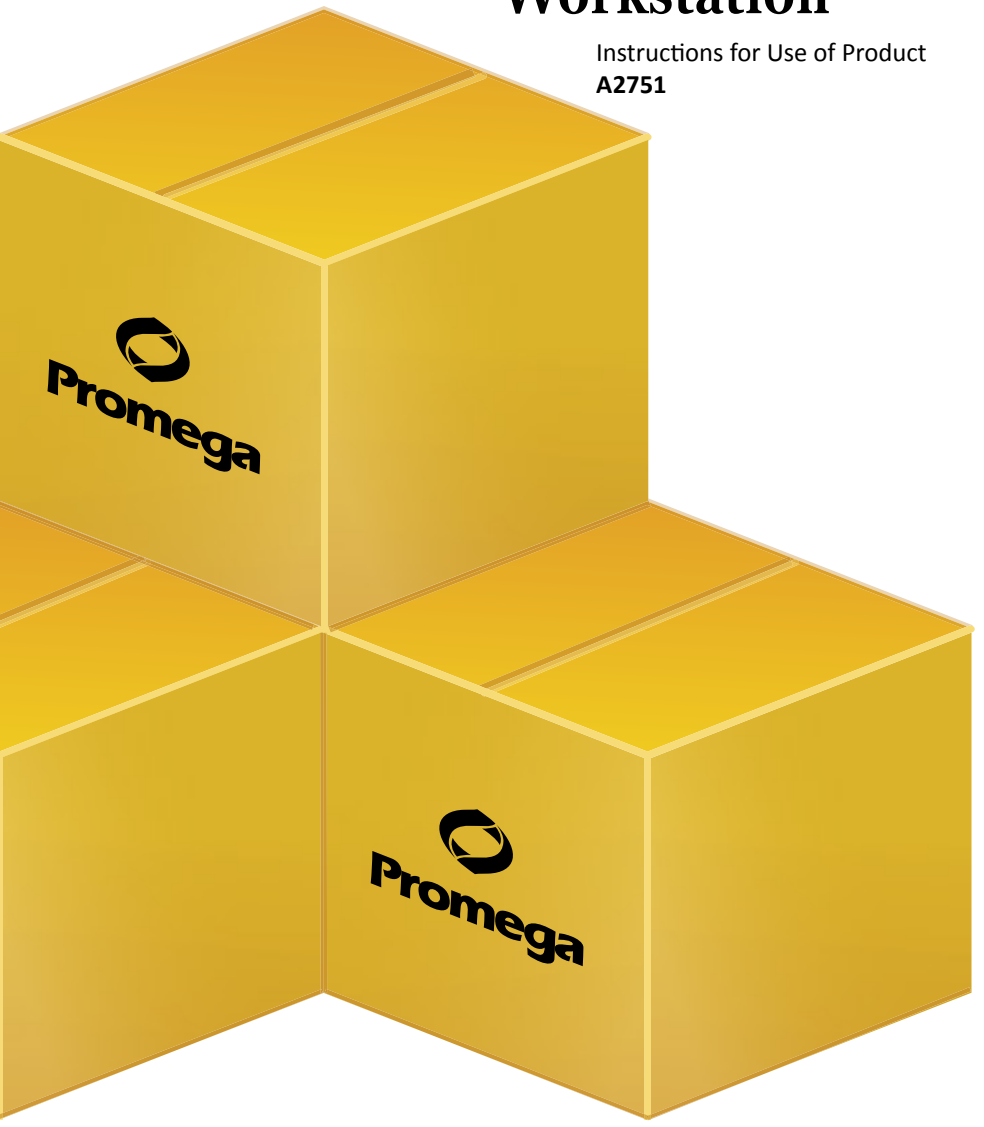
AUTOMATED PROTOCOL

Automated Genomic DNA Isolation from Mouthwash Samples using the Tecan Freedom EVO®-HSM Workstation

Instructions for Use of Product
A2751



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All technical literature is available at: www.promega.com/protocols/
Visit the web site to verify that you are using the most current version of this Automated Protocol.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

1. Description

The Tecan Freedom EVO®-HSM workstation automates the isolation of genomic DNA (gDNA) from mouthwash-collected buccal cells using the ReliaPrep™ Large Volume HT gDNA Isolation System chemistry. The automated protocol is preconfigured with the flexibility to meet the needs of high-throughput genomics workflows. The system processes any combination of sample volumes across the full input range of the chemistry, automatically metering reagents as appropriate for individual sample volumes. You need only to specify the number of samples to process and the desired elution volume. Additional options allow you to tailor the DNA purification process to meet your needs.

2. Product Components and Storage Conditions

PRODUCT	CAT.#
ReliaPrep™ Large Volume HT gDNA Isolation System	**A2751**

For Research Use. Each system contains sufficient reagents for 96 isolations from up to 10ml each. Includes:

- 23ml Proteinase K (PK) Solution
- 130ml Alkaline Protease (APA)
- 1,400ml Cell Lysis Buffer (CLD)
- 1,600ml Binding Buffer (BBA)
- 115ml ReliaPrep™ Resin
- 3,500ml Prepared Wash Buffer (WBC)
- 4 × 150ml Nuclease-Free Water

Storage Conditions: Store the ReliaPrep™ Large Volume HT gDNA Isolation System at room temperature (15–30°C). **Do not** refrigerate or freeze the reagents. Between uses, store on-deck reagents covered or capped at room temperature.

Note: Customers outside of the United States: Contact their local Promega Branch Office or Distributor for ordering information. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Available Separately

PRODUCT	SIZE	CAT.#
Tissue Lysis Buffer (TLA)	**500ml**	**A5091**
25mM Tris-HCl (pH 8.0)	**60ml**	**A2641**

Naming Conventions

Throughout this document, the solutions supplied with the ReliaPrep™ Large Volume HT gDNA Isolation System are referred to as follows:

- Proteinase K (PK) Solution = Proteinase K Solution
- Alkaline Protease (APA) = Alkaline Protease
- Cell Lysis Buffer (CLD) = Cell Lysis Buffer
- Tissue Lysis Buffer (TLA) = Tissue Lysis Buffer
- Binding Buffer (BBA) = Binding Buffer
- Prepared Wash Buffer (WBC) = Prepared Wash Buffer

3. Before You Begin

3.A. Compatible Sample Types

The method presented here is intended for processing buccal cells collected from 6–25ml mouthwash samples. Samples of varying volumes can be used as long as the sample volume in a single run is within the supported volume range. Samples should be free of food particles as they may block the vacuum tips. Compromised samples that have experienced suboptimal storage may be processed; however, the resulting DNA yield and quality may suffer depending on specific conditions. Contact Promega Technical Services if you have questions about compatible sample types: **techserv@promega.com**

3.B. Sample Collection Procedure

The sample collection procedure that follows was used during automated method development. You may test alternative sample collection methods, though results may vary. Samples were collected using Scope® Original Mint mouthwash. Bottled water results were similar to Scope® Original Mint mouthwash, but Listerine® was found to significantly reduce DNA yields. Sample contributors should rinse their mouths twice for 30 seconds with 10ml of mouthwash each time. Collect the spent mouthwash in a 50ml conical tube. Also collect any saliva or cells that can be removed from the inside of the mouth with the tongue. Pellet the buccal cells by centrifugation at 2,000 × *g* for 10 minutes. Resuspend the cells with 1ml of Nuclease-Free Water or Tissue Lysis Buffer (TLA) and store at –80°C until ready to process.

Samples may be stored for short periods at higher temperatures to allow for shipment. In this case, resuspend the cell pellet in TLA. We have observed no significant reduction in DNA yield for samples stored at room temperature for up to 7 days. After 7 days at 37°C, samples will yield over 80% of the DNA purified from samples stored under optimum conditions.

3.C. Sample Processing Options

Samples are processed in 50ml standard conical tubes preloaded in the HSM Instrument. If the samples were collected in 50ml conical tubes, then the resuspended pellets may be loaded directly into the HSM Instrument. We recommend processing buccal wash samples with RNase, but this option may be deactivated at run time.

3.D. Elution Options

DNA should be eluted using 25mM Tris-HCl (pH 8.0). You may select elution volumes between 500µl and 1,500µl. Eluting DNA in a smaller volume will increase the purified DNA concentration but also will result in lower total yield. Final eluted volumes of less than 1ml may result in poor resuspension of the ReliaPrep™ Resin, causing decreased performance and increased resin carryover into eluates.

3.D. Elution Options (continued)

All eluted DNA is transferred from the HSM to an intermediate 2.2ml, Square-Well Deep Well Plate (Cat.# V6781). If storage in TE is desired, the system can add concentrated EDTA to the intermediate plate. Following manual centrifugation, the system can transfer the samples to the final labware. The system supports a variety of final elution labware including 2D bar-coded tube racks, bar-coded plates and screw-capped tubes. Elution tubes should be compatible with standard Tecan tube carriers. Tube racks and plates should be ANSI/SLAS-compliant. If you select an elution volume greater than the maximum operating volume of the destination labware, the system will automatically override the selected volume. The elution volume will equal the maximum volume for the labware. You will be prompted to place the labware on the deck following centrifugation. Discuss final labware requirements with the technician during system installation.

3.E. DNA Quantitation and Evaluation

DNA quality and concentration can be determined by a variety of means including spectrophotometry, dye fluorescence, gel electrophoresis and quantitative PCR. It has been reported widely that different DNA quantitation methods frequently return dissimilar absolute quantitation values. We recommend that you use a consistent method for determining DNA quality and quantity throughout your procedure (i.e., use the same method for quantifying purified DNA and for qualifying DNA for input into your downstream application). Throughout this document, data are presented from NanoDrop® spectrophotometry.

4. Automated Processing

The Tecan Freedom EVO®-HSM Workstation provides several options for processing samples. These options are controlled through the TouchTools™ interface. All of the options described in Section 3 are accessible through this interface. Refer to the *Tecan Freedom EVO®-HSM Workstation Technical Manual*, #TM402, for instructions on using the TouchTools™ interface. For additional assistance with the user interface and processing options, contact Technical Services at: **techserv@promega.com**

4.A. Additional Materials

In addition to the Promega-supplied reagents, some additional supplies and reagents must be prepared and supplied by the user.

- **50% ethanol:** Combine equal volumes of 95–100% USP/ACS- or molecular biology-grade ethanol with molecular biology-grade water. Mix. **Note:** Using denatured ethanol that contains methanol or isopropanol may cause decreased DNA yield and purity.
- **100mM sodium hydroxide:** Prepare at least 300ml of 100mM sodium hydroxide in deionized water for each run. This is used to clean the system (e.g., Fisher Cat.# ss276-4).

4.A. Additional Materials (continued)

- **RNase A (optional):** Prepare an RNase A stock at 4mg/ml. RNase A is available from Promega (Cat.# A7974) prepared at the recommended concentration.
- **25mM Tris-HCl (pH 8.0):** This buffer is available from Promega (Cat.# A2641) or may be prepared by the user.
- **Concentrated EDTA (optional):** Promega offers 0.5M EDTA (pH 8.0; Cat.# A4231). Alternatively, prepare a concentrated stock of EDTA based on your laboratory's standard protocol. The stock should be concentrated between 10- and 20-fold higher than your desired final concentration. Select a concentration factor during the initial installation of your instrument to ensure that the correct dilution scheme is applied during processing.

The following Tecan consumables are required for a 32-sample extraction. We recommend using only filtered disposable tips to minimize cross-contamination risks.

Part Description	Quantity	Tecan Part#
LiHa Disposable Tips, 1000µl, Filtered	184 tips	10 612 513 or 10 612 555
Disposable Troughs for reagents, grey	6	10 613 049
25ml Disposable troughs	3	30 055 743

4.B. DNA Purification Procedure

The primary interface for the DNA purification method is Tecan TouchTools™ software. Using the interactive screens, you may select all of the user-configurable options for your run. For system operation instructions, refer to the *Tecan Freedom EVO®-HSM Workstation Technical Manual*, #TM402. From the available scripts, select Promega_Mouthwash_v1_1 and follow the graphical TouchTools™ interface to select all of your processing parameters. The interface will guide you through the complete system setup prior to beginning processing.

With all of the information entered, the system will begin the purification procedure. No additional input is required until the end of the method unless there are problems during sample transfer/detection or vacuum removal. If problems occur, refer to the Error Handling section of the *Tecan Freedom EVO®-HSM Workstation Technical Manual*, #TM402. After elution, you will be prompted to centrifuge the intermediate plate. This is necessary to remove any resin particles that may be present in the eluates. If you have chosen to leave your samples in the intermediate plate, you will not receive this prompt, as the method is now complete. Upon completion of the method, the system will prompt you to remove your samples, turn off the vacuum pump and store the remaining on-deck reagents.

4.C. Purification Steps

1. Automatic detection of sample volume and reagent volume calculation.
2. The HSM shakes at 500rpm during reagent addition.
3. Tissue Lysis Buffer (1ml) is added to each tube.
4. Proteinase K (60µl) is added to each tube.
5. After Proteinase K addition, the samples are incubated at 65°C for 30 minutes with shaking at 500rpm, followed by 10 minutes of shaking at 500rpm without heat. During this time the ReliaPrep™ Resin is kept in suspension through intermittent tip mixing.
6. Lysis Buffer (3ml) is added to each sample.
7. Binding Buffer (6ml) is added to each sample.
8. ReliaPrep™ Resin is thoroughly resuspended, and 300µl of resin is added to each sample. Binding of nucleic acid to the resin is accomplished through incubation with shaking for 20 minutes at 550rpm followed by magnetization for 20 minutes to collect the resin.
9. Waste from the lysis and binding is removed from each tube. After removal of waste, 1ml of Prepared Wash Buffer is added to the tube. This step is repeated until all tubes have had waste removed and wash added.
10. Samples are shaken at 500rpm for 2 minutes.
11. After shaking, the samples are mixed by pipetting to thoroughly disperse the resin.
12. **Recommended:** RNase A (60µl) is added to each sample.
13. The instrument adds 4ml of Prepared Wash Buffer while shaking. The wash is shaken at 500rpm for 2 minutes, then at 700rpm for 2 more minutes. Next, the resin is captured for 3 minutes.
14. Waste from the first wash is removed from each tube, then 1ml of Prepared Wash Buffer is added to the samples. Following this, the instrument adds 4ml of Prepared Wash Buffer while shaking. The wash is shaken at 500rpm for 2 minutes, then at 700rpm for 2 more minutes. Samples are then subjected to magnetic capture for 3 minutes.
15. Waste from the second wash is removed from each tube, then 4ml of 50% ethanol is added to the samples. The instrument shakes at 500rpm for 4 minutes. The samples are then subjected to magnetic capture for 3 minutes.
16. All waste is removed column by column, and the calculated amount of 25mM Tris-HCl (pH 8.0) is added to each tube. Samples are shaken at 500rpm for 3 minutes, then at 400rpm for 15 minutes at 70°C. Magnetic capture is performed for 3 minutes, and the eluates are transferred to the intermediate plate.
17. If elution to a final plate or tubes was selected the, the user is prompted to centrifuge the intermediate plate at 2,500 × *g* for 20 minutes to remove any particulates.
18. The intermediate plate is placed back on the instrument, and the eluates are transferred to the final elution labware.
19. The method is finished.

5. Developmental Results

The data described below are representative of multiple data sets obtained during development. For all samples shown, processing started with the samples in 50ml tubes. The resulting eluates were left in the intermediate plate for analysis.

5.A. Yields Across Individuals

During the development of the ReliaPrep™ buccal wash method, we processed samples from several individuals. We observed a range of DNA yields between individuals. Figure 1 shows the DNA yields for a representative group of individuals.

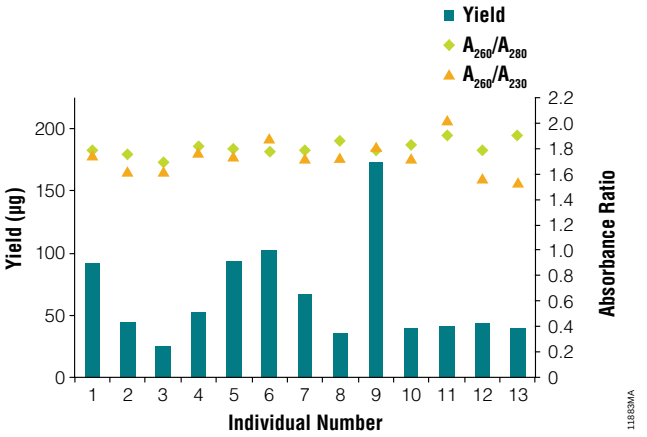

Figure 1. DNA yields and purities across multiple individuals. Each sample represents a single 25ml mouthwash sample collected from a different individual. DNA was eluted in a volume of 1,000µl. DNA yields and purities were were determined by NanoDrop® spectrophotometry.

5.B. Cross-Contamination Verification

This integrated system uses a series of cleaning steps to ensure that shared hardware components that contact samples do not present a cross-contamination risk. For a detailed description of the methods we have used to verify sample integrity on the Tecan Freedom EVO®-HSM Workstation, please refer to the *Verification of Sample Integrity for the Tecan Freedom EVO®-HSM Workstation Application Note*, #AN204, available at: **www.promega.com**

6. Troubleshooting

For questions not addressed here, please contact your local Promega Branch Office or Distributor. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

Symptoms	Possible Causes and Comments
Low DNA yield	Failure to resuspend the ReliaPrep™ Resin adequately before use. Thoroughly resuspend ReliaPrep™ Resin in the reagent bottle before dispensing for sample purification.
	Sample contained too few buccal cells. Saliva sample yields can vary across individuals by as much as 100µg.
	Sample size exceeded the processing capacity of the system. The ReliaPrep™ Large Volume HT gDNA Isolation System is optimized to purify DNA from 6–25ml of mouthwash. Processing samples that are outside of this volume range may result in reduced DNA yield and concentration.
	Successful use of the ReliaPrep™ Large Volume HT gDNA Isolation System depends on use of the correct reagents in the correct order. Check to ensure that all reagents were placed in correct positions on the instrument and that reagents were diluted correctly prior to use.
Degraded DNA	Nucleases were introduced during purification and by handling. Use nuclease-free plasticware or glassware. Use filter tips during all pipetting steps. Wear gloves at all times. Nucleases introduced after elution will degrade DNA. Use optional addition of concentrated EDTA to a final concentration of 1X in the final eluate to protect eluted DNA from nucleases.
	DNA was degraded before the purification process. If sample DNA was degraded before purification, the resulting purified DNA will also be degraded.
	DNA was eluted using Nuclease-Free Water, resulting in degradation. Use only 25mM Tris-HCl (pH 8.0) for eluting DNA.

6. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
ReliaPrep™ Resin in final eluate	Concentrated DNA solutions can be viscous. Additional time may be required to capture the ReliaPrep™ Resin from such viscous solutions. Remove residual resin from the eluted DNA by performing the indicated centrifugation.
Reduced DNA purity	A reduction in DNA purity as measured by spectroscopic ratios or downstream assay results is often the result of incomplete lysate removal. Any physical adjustments of the robotic system can influence the efficiency of lysate removal. Contact **techserv@promega.com** before attempting any adjustments to the robotic system.
Instrument error messages	If the system reports insufficient volume in a reagent trough, verify that the correct trough type is used for the reagent and add more reagent to the trough. For all other errors contact Promega Technical Services. Make a note of the specific error message and time. You will need the detailed EVOware® logs that can be found at: **C:\ProgramData\Tecan\EVOware\AuditTrail\log**. The log files use the naming convention **EVO_yyyymmdd_hhmmss.log**, where the timestamp represents the date of file creation.

7. Related Products

PRODUCT	SIZE	CAT.#
HSM 2.0 Instrument	1 each	A2715
RNase A Solution	5ml	A7974
Tecan Freedom EVO®-HSM Workstation		Contact Tecan
Cell Lysis Buffer (CLD)	1,400ml	A1731
Binding Buffer (BBA)	1,600ml	A1741
ReliaPrep™ Resin	115ml	A1752
Prepared Wash Buffer (WBC)	3,500ml	A2681
Proteinase K (PK) Solution	23ml	A5051
Tissue Lysis Buffer (TLA)	500ml	A5091
Bottle for 50% Ethanol	1 each	A2691
Integrated Reagent Caps	4/pk	A2701
10mM EDTA (pH 8.0)	10ml	A2631
25mM Tris-HCl (pH 8.0)	60ml	A2641

8. Summary of Changes

The following change was made to the 7/17 revision of this document:

Related Product information for the Tecan Freedom EVO®-HSM Workstation was changed to "Contact Tecan."