AUTOMATED PROTOCOL

Automated Genomic DNA Isolation from Whole Blood using the Tecan Freedom EVO®-HSM Workstation

Instructions for Use of Product **A2751**

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

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1. Description

The Tecan Freedom EVO®-HSM workstation automates the isolation of genomic DNA (gDNA) from whole blood 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 your local Promega Branch Office or Distributor for ordering information. Contact information available at: **www.promega.com**. E-mail: **techserv@promega.com**

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
- Binding Buffer (BBA) = Binding Buffer
- Prepared Wash Buffer (WBC) = Prepared Wash Buffer

3. Before You Begin

3.A. Compatible Sample Types

The methods presented here are intended for processing whole blood from 1–10ml. Any combination of blood samples can be processed within the supported volume range in a single run. The blood may be collected in most common anti-coagulants. Blood may be fresh or frozen. Clotted blood is not recommended as it may block the vacuum aspiration tips or sample transfer tips and may result in colored eluates with low purity. Samples should be able to pass through a standard 10ml serological pipet. Compromised blood that has 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 Processing Options

Samples are processed in 50ml standard conical tubes preloaded in the HSM. The system can automatically transfer samples from a variety of labware including vacuum collection tubes if you do not wish to transfer the samples manually. Selecting automated sample transfer will increase the total processing time by up to 1 hour. When transferring from source labware, the system can transfer the entire contents of the tubes or transfer up to a maximum specified volume. Only a single labware type may be selected for any run. If using Vacutainer® tubes, sample tubes may have either 13mm or 16mm diameters, but only one tube diameter can be used for a single run. Discuss sample input labware options with the technician during system installation. Samples can be treated with RNase A, if desired (optional reagent purchased separately).

3.C. Elution Options

Elute DNA using the included Nuclease-Free Water. 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 for high-volume samples, causing decreased performance and increased resin carryover into eluates. You have the option of scaling elution volumes to the initial sample volume. In this case samples between 1ml and 3ml will be eluted into 500µl of water, and 10ml samples will be eluted into 1,500µl of water. Sample elution volumes will be adjusted linearly for samples between 3ml and 10ml.

All eluted DNA is transferred from the HSM to an intermediate deep 96-well plate. If storage in TE is desired, the system can add concentrated Tris-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 be the same as the maximum volume for the labware. You will be prompted to place the elution labware on the deck following centrifugation. Discuss final labware requirements with the technician during system installation.

3.D. 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 and QuantiFluor® dsDNA System.

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).
- **RNase A (optional):** Prepare an RNase A stock at 4mg/ml. RNase A is available from Promega (Cat.# A7974) prepared at the recommended concentration.
- **Concentrated Tris-EDTA (TE; optional):** Promega offers 20X TE Buffer (pH 7.5; Cat.# A2651). Alternatively, prepare a concentrated stock of TE depending on your lab preferences. The stock should be concentrated between 10- and 20-fold higher than your desired final concentration. It is important to 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_Blood_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 prior to automated eluate transfer to the final elution labware. 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. **Optional:** Sample transfer from primary tubes.
2. Automatic detection of sample volume and reagent volume calculation. Note that the reagent volumes for samples less than 3ml will be equivalent to 3ml of whole blood.
3. The HSM shakes at 500rpm during reagent addition.
4. Proteinase K (0.02 volumes) is added to each tube.
5. Alkaline Protease (0.125 volumes) is added to each sample.
6. One volume of Lysis Buffer is added to each sample.
7. After Lysis Buffer 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.
8. Binding Buffer (1.2 volumes) is added to each sample.
9. ReliaPrep™ Resin is thoroughly resuspended, and 0.1 volume 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 10–20 minutes (based on maximum original input sample volume) to collect the resin.
10. Waste from the lysis and binding is removed from each tube. After removal of waste, 1–3ml of Prepared Wash Buffer, based on original sample volume, are added to the tube. This step is repeated until all tubes have had waste removed and wash added.
11. Samples are shaken at 500rpm for 2 minutes.
12. After shaking, the samples are mixed by pipetting to thoroughly disperse the resin.

4.C. Purification Steps (continued)

13. **Optional:** RNase A (0.02 volumes) is added to each sample.
14. The instrument adds additional Prepared Wash Buffer in the range of 4.4–9ml, based on original sample volume, 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.
15. 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 additional Prepared Wash Buffer in the range of 4.4–9ml, based on original sample volume, 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.
16. Waste from the second wash is removed from each tube, then 4.4–9ml of 50% ethanol, based on original sample volume, is added to the samples. The instrument shakes at 500rpm for 4 minutes. The samples are then subjected to magnetic capture for 3 minutes.
17. All waste is removed column by column, and the calculated amount of Nuclease-Free Water 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.
18. 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.
19. The intermediate plate is placed back on the instrument, and the eluates are transferred to the final elution labware.
20. 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. Automated Versus Manual Purification Yield Comparison

The DNA yields obtained with the ReliaPrep™ automated DNA purification system correlate linearly with sample input volume across the full sample input range. The samples shown in Figure 1 were taken from a single individual. Each sample was processed using either Wizard® Genomic precipitation-based chemistry or the automated ReliaPrep™ System. Samples have an average A_{260}/A_{230} ratio of 2.1 and average A_{260}/A_{280} ratio of 2.0.

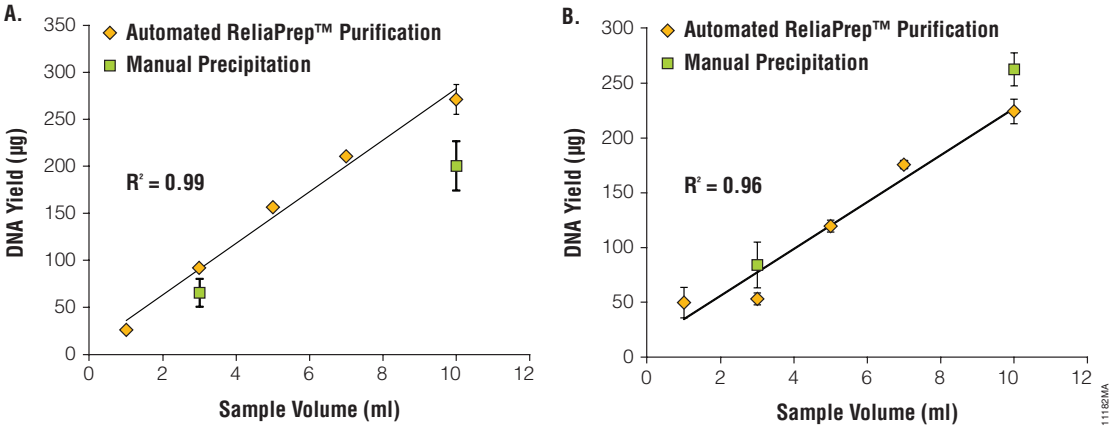

Figure 1. Automated DNA yields across the sample volume range of whole blood. Whole blood samples were prepared from a single individual. Each point is the mean of quadruplicate values with error bars of 1 standard deviation. **Panel A.** DNA yields as determined by NanoDrop® spectrophotometer. **Panel B.** DNA yields as determined by QuantiFluor® dsDNA System.

5.B. Yields Across Individuals

During the development of the ReliaPrep™ blood method, we processed samples from several individuals. Figure 2 shows the DNA yield per white blood cell (WBC).

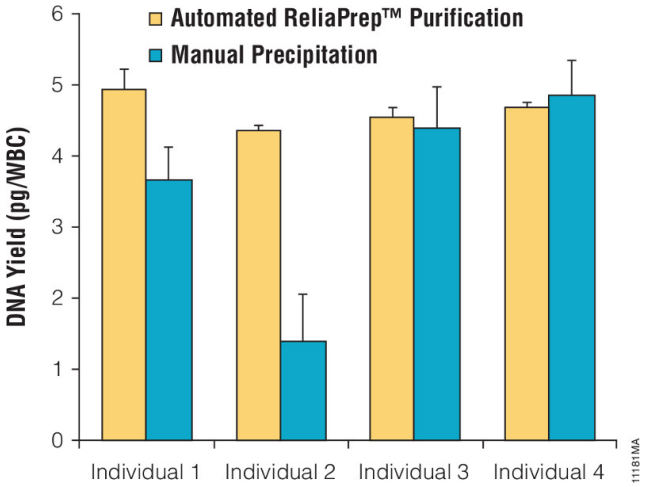

Figure 2. Normalized DNA yields across multiple individuals. DNA yields normalized based on the white blood cell counts for each individual. Individual 1 is from the same individual as in Figure 1. Each bar represents the mean of quadruplicate samples with error bars of one standard deviation.

5.C. 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 white blood cells per milliliter of blood. DNA yield depends on the amount of white blood cells in the starting material. Blood samples with low white cell counts will have reduced yields because of the low sample input.
	Sample size exceeded the processing capacity of the system. The ReliaPrep™ Large Volume HT gDNA Isolation System is optimized to purify DNA from 1–10ml of whole blood. Processing blood samples that are outside of this volume range may result in reduced DNA yield and concentration. Exceeding the 10ml limit will cause the required processing volumes to exceed the maximum working volume of a 50ml tube. The ReliaPrep™ Large Volume HT gDNA Isolation System is optimized for normal, healthy-adult whole blood samples, which generally contain between 4.5×10^6 and 1×10^7 white cells/ml. Whole blood samples containing more white cells per milliliter may cause slower capture of the ReliaPrep™ Resin during the purification process, resulting in reduced yield.
	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 TE Buffer 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.

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 additional 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
20X TE Buffer (pH 7.5)	25ml	A2651
Tecan Freedom EVO®-HSM Workstation		Contact Tecan
Alkaline Protease (APA)	130ml	A1721
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
Nuclease-Free Water	500ml	P1197
	1,000ml	P1199
Tissue Lysis Buffer (TLA)	500ml	A5091
Bottle for 50% Ethanol	1 each	A2691
Integrated Reagent Caps	4/pk	A2701

8. Summary of Changes

The following change was made to the 1/18 revision of this document:

Corrected Figure 2.