AUTOMATED PROTOCOL

Automated Genomic DNA Isolation from Low Yielding Buffy Coat Samples 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

1. Description

The Tecan Freedom EVO®-HSM workstation fitted with the Low Volume Adapter accessory automates the isolation of genomic DNA (gDNA) from low-cell-count buffy coat samples using the ReliaPrep™ Large Volume HT gDNA Isolation System chemistry. The automated protocol is pre-configured with the flexibility to meet the needs of high-throughput genomics workflows. The system processes any combination of sample inputs across the full input range of the chemistry, automatically metering reagents as appropriate for individual sample volumes. Users need only to specify the number of samples to process and the desired elution volume. Additional options allow the users to tailor the DNA purification process to meet their 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

Available Separately

PRODUCT	CAT.#
Low Volume Adapter System and Methods	**XAT1020**

For Research Use.

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 should contact their 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
- Low Volume Adapter (LVA) = Low Volume Adapter

3. Before You Begin

3.A. Additional Hardware Requirements

Samples are processed using the Tecan Freedom EVO®-HSM workstation. Processing low-cell-count buffy coat samples requires the Low Volume Adapter (LVA) and MagnaBot® 96 Magnetic Separation Device, available as Cat.# XAT1020, Low Volume Adapter System and Methods. Processing samples using the methods described here without the Low Volume Adapter risks damage to the workstation and loss of sample. The Low Volume Adapter and MagnaBot® 96 Magnetic Separation Device must be installed by a qualified Promega representative. Contact your local Promega branch office for information on purchasing a Low Volume Adapter.

3.B. Compatible Sample Types

The method presented here is intended for processing buffy coat samples containing 2×10^6–2.5×10^7 white blood cells. While buffy coat samples in the specified white blood cell input range can be prepared from any starting volume of blood, the volume of the actual buffy coat samples should be 1ml or less. Any combination of buffy coat sample volumes up to 1ml can be processed within the supported input range in a single run. The starting blood samples may be collected in most common anti-coagulants. Samples may be fresh or frozen, but buffy coat samples should be collected from fresh blood. Clotted samples are not recommended as the clots 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 1ml pipette tip. Compromised samples that have been stored under suboptimal conditions may be processed; however, the resulting DNA yield and quality may suffer. Contact Promega Technical Services if you have questions about compatible sample types: **techserv@promega.com**

3.C. Sample Processing Options

Samples are processed in 14ml round-bottom tubes (e.g., Becton, Dickinson and Company Cat.# 352018) preloaded in the HSM. If you do not wish to transfer the samples manually, the system can automatically transfer samples from a variety of labware including vacuum collection tubes. Selecting automated sample transfer will increase the total processing time by up to 20 minutes. During automated transfer, the system will transfer a maximum of 1.0ml of sample even if more is present in the labware. Samples can be treated with RNase A, if desired (optional reagent purchased separately; see Section 4.A, Additional Materials).

3.D. Elution Options

DNA should be eluted using the included Nuclease-Free Water. You may select elution volumes between 100µl and 500µl. Eluting DNA in a smaller volume will increase the purified DNA concentration but also will result in lower total yield.

All eluted DNA is transferred from the HSM to an intermediate 96-well, deep-well plate. If storage in TE is desired, the system can add concentrated Tris-EDTA (optional reagent purchased separately; see Section 4.A, Additional Materials) to the intermediate plate. Following final resin capture, the system can transfer the samples to the final labware. The system supports a variety of final elution labware including 2D barcoded tube racks, barcoded 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. 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, use of an intercalating fluorescent dye such as QuantiFluor® dsDNA Dye, 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).
- **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 risk.

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

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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_LVA_Buffy_v1_1 and follow the graphical TouchTools™ interface to select 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. If you have chosen to to elute your samples into tubes, you will be prompted to load your elution tubes towards the end of processing. 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.
3. Proteinase K (40µl) is added to each tube.
4. Alkaline Protease (200µl) is added to each sample.
5. One milliliter of Lysis Buffer is added to each sample.
6. After Lysis Buffer addition, the samples are incubated at 65°C for 30 minutes with shaking at 750rpm, followed by 10 minutes of shaking at 500rpm without heat. During this time the ReliaPrep™ Resin is kept in suspension through intermittent tip mixing.
7. Binding Buffer (1.2ml) is added to each sample.
8. ReliaPrep™ Resin is thoroughly resuspended, and 100µl of resin is added to each sample. Binding of nucleic acid to the resin is accomplished through incubation with shaking for 20 minutes at 775rpm 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, 1–1.5ml 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.
10. **Optional:** RNase A (40µl) is added to each sample.
11. Samples are shaken at 750rpm for 2 minutes.

4.C. Purification Steps (continued)

12. After shaking, the samples are mixed by pipetting to thoroughly disperse the resin. Following this, the instrument adds additional Prepared Wash Buffer (1ml). The wash is shaken at 750rpm for 2 minutes. Next, the resin is captured for 30 seconds.
13. 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 (1ml). The wash is shaken at 750rpm for 3 minutes. Samples are then subjected to magnetic capture for 30 seconds.
14. Waste from the second wash is removed from each tube, then 1ml of 50% ethanol is added to the samples. The instrument shakes at 750rpm for 4 minutes. The samples are then subjected to magnetic capture with heating at 70°C for 30 seconds.
15. All waste is removed column by column, and the tubes are allowed to dry for 20 minutes.
16. The calculated amount of Nuclease-Free Water is added to each tube for elution. Samples are shaken at 600rpm for 20 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 system will pause to allow final resin capture.
18. Following final resin capture, the eluates are transferred to the final elution labware.
19. The method is finished.

5. Developmental Results

The data shown below are representative of multiple data sets obtained during development. For all samples shown, processing started with the samples in 14ml tubes. The resulting eluates were left in the intermediate plate for analysis.

5.A. Automated Versus Manual Purification Yield Comparison

The DNA concentrations obtained with the ReliaPrep™ automated DNA purification system correlate linearly with WBC input across the full sample input range. The samples shown in Figure 1 were taken from a single individual. Defined WBC samples were produced by mixing pooled buffy coat samples and WBC-depleted blood prepared from normal whole blood. The WBC count was measured by HemoCue® WBC System. Each sample is a 1ml aliquot of the mixed samples. Samples have an average A_{260}/A_{230} ratio of 2.1 and average A_{260}/A_{280} ratio of 2.0.

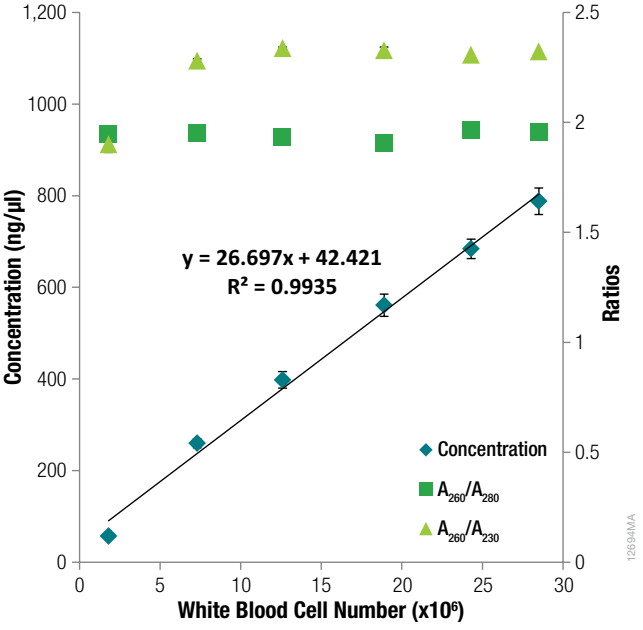

Figure 1. Automated DNA yield concentration across the WBC input range. Samples were prepared from a single individual and eluted in 200µl of Nuclease-Free Water. The buffy coat samples of specified WBC count were produced by mixing high-WBC buffy coat samples with WBC-depleted blood. Each point is the mean of quadruplicate values with error bars of 1 standard deviation. DNA concentrations were determined by NanoDrop® spectrophotometer.

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	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 starting blood sample. DNA yield depends on the amount of white blood cells in the starting material. Buffy coat samples with extremely low white cell counts will have reduced yields because of the low sample input.
	Sample size exceeded the processing capacity of the system. This ReliaPrep™ Large Volume HT gDNA Isolation System protocol is optimized to purify DNA from 2×10^6–2.5×10^7 white blood cells. Processing buffy coat samples outside of this white blood cell count range may result in reduced DNA yield and concentration. Exceeding the 2.5×10^7 white blood cells limit could result in reduced yield and poor sample purity.
	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.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
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 the 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.
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 centrifugation (10 minutes at 2500 × *g*).
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 or poor wash mixing.
	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_yyyym-mdd_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
Bottle for 50% Ethanol	1 each	A2691
Low Volume Adapter System and Methods	1 each	XAT1020
Integrated Reagent Caps	4/pk	A2701

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.”