TECHNICAL MANUAL

ReliaPrep™ Large Volume HT gDNA Isolation System

Instructions for Use of Product
A2751

Revised 11/18
TM341

ReliaPrep™ Large Volume HT gDNA Isolation System

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 Technical Manual.
E-mail Promega Technical Services if you have questions on use of this system: techserv@promega.com

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1. Description

The ReliaPrep™ Large Volume HT gDNA Isolation System[a] isolates genomic DNA (gDNA) from blood samples ranging from 1ml to 10ml of blood in a scalable format. The chemistry eliminates tedious centrifugation steps as well as the use of hazardous chemicals, which are inherent in precipitation-based chemistries. The system has been automated on liquid-handling workstations, allowing walkaway purification of genomic DNA from 1–10ml of whole blood, regardless of sample storage or shipping conditions. The system is scalable and uses only the amount of reagents required to process each sample based on sample volume, maximizing efficiency and value per prep.

Sample processing is simplified through the use of the HSM 2.0 Instrument (Cat.# A2715), which heats, shakes and magnetizes sample tubes in one position throughout the extraction process from lysis to elution. This system removes resource limitations from sample processing and increases automated method robustness and reliability. Genomic DNA yields from normal 10ml whole blood samples are typically 200–400µg (depending on white blood cell count) in a final eluted volume of 1ml. Recovered DNA exhibits good purity with A_{260}/A_{280} ratios greater than 1.7 and A_{260}/A_{230} ratios between 1.5 and 2.2. The ReliaPrep™ Large Volume HT gDNA Isolation System, in combination with the HSM 2.0 Instrument, offers a dependable and efficient option for automated isolation of high-quality DNA from large-volume blood samples.

Hardware Requirements

For manual DNA purification from 1–32 blood samples (1–10ml each), the hardware listed below is required.

PRODUCT	QUANTITY	CAT.#
HSM 2.0 Instrument (processes up to 32 samples per instrument)	**1**	**A2715**

The HSM 2.0 Instrument is supplied with one HSM 2.0 Tube Rack and one HSM 2.0 Tube Rack Stand. For people who wish to purchase additional HSM 2.0 Tube Racks and HSM 2.0 Tube Rack Stands, these items are available separately.

AVAILABLE SEPARATELY	CAT.#
HSM 2.0 Tube Rack	**A2713**
HSM 2.0 Tube Rack Stand	**A2714**

2. Product Components and Storage Conditions

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

Each system contains sufficient reagents for 96 isolations from up to 10ml each or 320 × 1–3ml in a scalable format. 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 any of the reagents.

Naming Conventions: Throughout this Technical Manual, the solutions supplied with the ReliaPrep™ Large Volume HT gDNA Isolation System are referred to as follows: Proteinase K (PK) Solution as Proteinase K Solution, Alkaline Protease (APA) as Alkaline Protease, Cell Lysis Buffer (CLD) as Cell Lysis Buffer, Binding Buffer (BBA) as Binding Buffer, Prepared Wash Buffer (WBC) as Prepared Wash Buffer.

3. General Considerations

3.A. Comparison with Organic- and Precipitation-Based Methods

The ReliaPrep™ Large Volume HT gDNA Isolation System addresses the challenges of traditional genomic DNA purification methods that rely on centrifugation-based organic or precipitation chemistries by isolating genomic DNA from the entire lysed whole blood sample. The ReliaPrep™ Large Volume HT gDNA Isolation System efficiently purifies genomic DNA regardless of whether blood samples are fresh or frozen.

3.B. Sample Processing Capacity

Genomic DNA yield depends on 1) volume of whole blood processed and 2) number of white blood cells/ml. The ReliaPrep™ Large Volume HT gDNA Isolation System purifies genomic DNA from up to 10ml of whole blood in one purification procedure. This volume limit assumes that the average number of white blood cells per 1ml of whole blood from a normal healthy adult is between 4.5×10^6 and 1.1×10^7 cells/ml. Therefore, the cell number limit of the ReliaPrep™ Large Volume HT gDNA Isolation System is approximately 1×10^8 leukocytes. Exceeding the recommended volume or cell number may result in clumping of the ReliaPrep™ Resin and reduced gDNA yield and quality.

3.C. Elution

The recommended elution volumes in the ReliaPrep™ Large Volume HT gDNA Isolation System protocol may be adapted to accommodate downstream applications that require a specific DNA concentration. The recommended elution volume range is 700–1,700µl. The final recovered volume will be approximately 200µl less than the volume of Nuclease-Free Water added, for a final elution volume of 500–1,500µl. Eluting DNA in a smaller volume will increase the purified DNA concentration but also will result in lower total yield. In addition, elution volumes of less than 1ml may result in poor resuspension of the ReliaPrep™ Resin, causing decreases in performance and increased resin carryover into eluates. For optimal elution of genomic DNA from the ReliaPrep™ Resin, add room-temperature Nuclease-Free Water to the ReliaPrep™ Resin, mix and heat with the HSM 2.0 Instrument. Failure to heat after adding the Nuclease-Free Water will decrease DNA yield and concentration. For optimal elution, the ReliaPrep™ Resin must be heated and mixed vigorously during mix steps to efficiently release genomic DNA.

3.D. Additional Sample Types

The ReliaPrep™ Large Volume HT gDNA Isolation System has been adapted to isolate gDNA from additional sample types including blood fractions and saliva. For a complete list of compatible sample types and protocols, visit: **www.promega.com/products/biobanking/**

3.E. Manual Versus Automated Processing

For more information about the HSM 2.0 Instrument, see the *HSM 2.0 Instrument Technical Manual* #TM389 at: **www.promega.com/protocols/**

Manual Processing

The HSM 2.0 Instrument is controlled by software installed on a computer with the Windows® 7 operating system with methods to process 1–10ml of blood. These methods guide the user through the purification process with specific instructions on reagent addition and waste removal. Solutions are typically added with a pipette and aspirated using a vacuum aspirator.

Automated Processing

Automated processing is available using a liquid-handling workstation and integrated HSM 2.0 Instrument. Automated processing provides scalability to adapt easily to daily needs; the instrument senses the sample volume in each tube and scales reagent volumes appropriately without user intervention. The method will process variable sample volumes within a single run as well as variable batch sizes between runs.

For more information about liquid-handling options contact Promega at: **techserv@promega.com**

4. Genomic DNA Purification Procedure

This overview describes the liquid-handling and purification steps required to isolate genomic DNA from whole blood using the ReliaPrep™ Large Volume HT gDNA Isolation System. This protocol can be performed manually using the preloaded methods in the HSM 2.0 Software (Section 4.B) or an automated liquid-handling workstation.

4.A. Reagent Preparation

47.5–50% ethanol: Combine equal volumes of 95–100% USP/ACS- or Molecular Biology-grade ethanol with Molecular Biology-grade water. Mix.

! Use of ethanol that contains methanol or isopropanol may cause decreased DNA yield and purity.

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4.B. Manual DNA Purification

Materials to Be Supplied by the User

- HSM 2.0 Instrument (Cat.# A2715) with HSM 2.0 Tube Rack, HSM 2.0 Tube Rack Stand and computer with HSM 2.0 Instrument software installed (see *HSM 2.0 Instrument Operating Manual* #TM389 for details)
- 47.5–50% ethanol, at room temperature, prepared using Molecular Biology-grade water (see Section 4.A)
- 50ml conical tubes (one per sample, e.g., Corning Cat.# 430291)
- 10ml pipettes and pipette aid
- **Optional:** A repeater pipette (Gilson or Eppendorf) for adding reagents to larger numbers of samples

Be sure to remove all lysate, Prepared Wash Buffer and ethanol from the tubes in Steps 7, 9, 11 and 13. Failure to remove all lysate, Prepared Wash Buffer and ethanol will result in decreased purity of the eluted DNA.

1. Place the HSM 2.0 Tube Rack on the HSM 2.0 Tube Rack Stand.
2. Place one empty, uncapped, 50ml conical tube per sample into the HSM 2.0 Tube Rack. Add blood samples to the uncapped 50ml tubes.

! The ReliaPrep™ Large Volume HT gDNA Isolation System is designed to process whole blood in uncapped tubes. Do not dilute blood prior to processing.

3. Transfer the HSM 2.0 Tube Rack to the HSM 2.0 Instrument.
4. Launch the HSM 2.0 Instrument software, and select the appropriate protocol for the desired sample type. Enter the blood volume, and the software will provide reagent volumes based on the volume provided. If you are processing multiple blood volumes refer to Table 1 for reagent volumes.

Table 1. Reagent Volumes (ml) Across Multiple Sample Volumes.

	Sample Volume (ml)							
Reagent (per sample)	**≤3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Proteinase K	0.060	0.080	0.100	0.12	0.14	0.16	0.180	0.20
RNase (Optional)	0.060	0.080	0.100	0.12	0.140	0.16	0.180	0.20
Alkaline Protease	0.375	0.500	0.625	0.75	0.875	1.00	1.125	1.25
Lysis Buffer	3.00	4.00	5.00	6.00	7.00	8.00	9.00	10.00
Binding Buffer	3.60	4.80	6.00	7.20	8.40	9.60	10.80	12.00
Prepared Wash Buffer	5.00	5.60	6.10	6.70	7.30	7.90	8.40	9.00
50% Ethanol	4.00	4.60	5.10	5.70	6.30	6.90	7.40	8.00

Note that samples with volumes less than 3ml are processed using the reagent volumes for 3ml of whole blood.

5. Add the volume of Proteinase K Solution displayed on-screen to each tube.
6. **Optional:** Add the indicated volume of RNase A indicated on screen to each tube.
7. Select "OK" to start shaking samples at 500rpm for 1 minute. After the beep, add the volume of Alkaline Protease indicated on screen to each tube. Select "OK" to start shaking samples at 500rpm for 1 minute.
8. Add the appropriate volume of Lysis Buffer to each sample. After Lysis Buffer addition, the HSM 2.0 Instrument incubates the samples at 65°C for 30 minutes with shaking at 500rpm, followed by 10 minutes of shaking at 500rpm without heat.
9. Add the appropriate volume of Binding Buffer to each sample. Select "OK" to start shaking samples at 550rpm for 3 minutes. After shaking, thoroughly resuspend the ReliaPrep™ Resin, and add the appropriate volume of resin to each sample as shown on screen. Select "OK" to start shaking samples at 550rpm for 20 minutes.

! Thoroughly resuspend the ReliaPrep™ Resin before dispensing.

10. After the shaker stops, the HSM 2.0 Instrument collects the ReliaPrep™ Resin at the side of the tube for 20 minutes. When prompted by the instrument, slowly aspirate and dispose of the lysate in the tube.

! Remove all lysate from the tubes before adding wash buffer in Step 11. After the initial removal, perform an additional aspiration to ensure that all lysate is removed.

Note: Rapid aspiration of the lysate may result in loss of the resin.

11. Add the amount of Prepared Wash Buffer indicated on screen to each tube. Press "Enter" to start samples shaking at 500rpm for 2 minutes. The ReliaPrep™ Resin should dislodge from the side of the tube during this shaking step.
12. After shaking for 2 minutes, the HSM 2.0 Instrument prompts you to mix the samples. Pipet the samples at least

! 10 times to thoroughly disperse the resin. Following this, the instrument will shake again for 2 minutes to wash the resin.

13. The HSM 2.0 Instrument collects the ReliaPrep™ Resin for 3 minutes. Slowly aspirate the fluid from each tube when prompted by the instrument.

! Remove all wash buffer before adding fresh Prepared Wash Buffer in Step 14. After the initial removal, perform an additional aspiration to ensure that all Wash Buffer is removed.

14. Add the amount of Prepared Wash Buffer indicated on screen to each tube. Select "OK" to start samples shaking at 500rpm for 3 minutes, then shaking for 3 minutes at 700rpm.

4.B. Manual DNA Purification (continued)

15. The shaker will stop, and the HSM 2.0 Instrument collects the ReliaPrep™ Resin at the side of the tube for 3 minutes. Slowly aspirate the fluid from each tube when prompted by the instrument.

Remove all Wash Buffer before adding ethanol in Step 16. After the initial removal, perform an additional aspiration to ensure that all Wash Buffer is removed.

16. Add the amount of 47.5–50% ethanol shown on screen to each sample. Select "OK" to start samples shaking samples at 500rpm for 4 minutes.

17. The shaker will stop, and the HSM 2.0 Instrument collects the ReliaPrep™ Resin at the side of the tube for 3 minutes. Slowly aspirate the fluid from each tube when prompted by the instrument.

    Remove all ethanol from the tubes before adding Nuclease-Free Water in Step 18. After the initial removal, perform an additional aspiration to ensure that all ethanol is removed.

18. Add 1.5ml of Nuclease-Free Water to each tube. The HSM 2.0 Instrument first disperses the resin in the Nuclease-Free Water by shaking for 3 minutes at 600rpm and then incubates the samples at 70°C for 20 minutes with shaking at 400rpm.

    Note: A volume of 1.5ml of Nuclease-Free Water will produce a final eluted volume of approximately 1.3ml. The final recovered volume will be approximately 200µl less than the volume of Nuclease-Free Water added. Adding less Nuclease-Free Water will reduce the total eluted volume, creating eluates of higher concentration, but may decrease total yields. Do not elute with less than 700µl or more than 1.7ml of Nuclease-Free Water.

19. The HSM 2.0 Instrument collects the ReliaPrep™ Resin at the side of each tube for 5 minutes. Transfer each eluate to a 1.5ml tube or 96-well plate.

    Note: Removing all ReliaPrep™ Resin from the eluate requires a centrifugation step (2 minutes at maximum speed in a microcentrifuge if eluates are in tubes or 10 minutes at 2,500 × *g* if eluates are in a 96-well plate). We recommend adding concentrated TE buffer (10X or 20X) to eluted samples to a final concentration of 1X TE buffer for long-term storage.

5. Quantitation and Analysis of Isolated Genomic DNA

DNA quality and concentration can be determined by a variety of means including spectrophotometry, dye fluorescence, gel electrophoresis, and quantitative PCR. Different DNA quantitation methods frequently return dissimilar absolute quantitation values. We recommend that users select analysis methods for DNA quality and quantity that best predict success for the intended downstream applications.

A_{260}/A_{280} ratio can be used to determine DNA purity (with a number of important limitations [1–3]). An A_{260}/A_{280} ratio between 1.7 and 2.0 is generally accepted as representative of a high-quality DNA sample. The ratio can be calculated after subtracting the non-nucleic acid absorbance at A_{320}.

$$\text{DNA purity } (A_{260}/A_{280}) = \frac{(A_{260}\text{ reading} - A_{320}\text{ reading})}{(A_{280}\text{ reading} - A_{320}\text{ reading})}$$

Note: Many spectrophotometers automatically subtract the absorbance at a reference value around 340nm from the absorbance at 230nm, 260nm and 280nm before reporting these values and ratios. Before performing this calculation, check with your spectrophotometer user manual to determine whether your instrument performs this calculation.

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	Insufficient mixing during elution. During successful elution, the ReliaPrep™ Resin is thoroughly resuspended during the mixing step, and the elution solution is a homogeneous dull-brown color. During poor elution, the ReliaPrep™ Resin remains in a loose clump, swirling at the bottom of the tube during the shaking steps. Be sure that the ReliaPrep™ Resin is thoroughly resuspended during the elution step.
	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 cells per 1ml of blood. DNA yield depends on the amount of starting material. Blood samples with low white cell counts will have reduced yields due to low sample input.
	Elution volume was too low. Using a smaller elution volume will increase purified DNA concentration but also will decrease total yield. The final eluted volume needs to be at least 1ml for the ReliaPrep™ Resin to be captured by the magnets within the HSM 2.0 Instrument.

6. Troubleshooting (continued)

Symptoms	Possible Causes and Comments
Low DNA yield (continued)	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 range will result in reduced DNA yield and concentration. Exceeding the 10ml limit will cause the required processing volumes to exceed the maximum volume of a 50ml tube. The ReliaPrep™ Large Volume HT gDNA Isolation System is optimized for whole blood samples that contain between 4.5×10^6 and 1.1×10^7 white cells/ml. Whole blood samples containing more white cells per 1ml may result in poor performance.
	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 protocol steps were followed correctly and that the correct reagents were used at each step. This ensures optimal purification of genomic DNA away from sample contaminants.
Purity (A_{260}/A_{280} or A_{260}/A_{230}) of eluted DNA is low	Incomplete lysis of red blood cells. Failure to add sufficient volume of Proteinase K Solution, Alkaline Protease solution or Cell Lysis Buffer will result in incomplete lysis and digestion of cells and proteins. Try using Proteinase K and Alkaline Protease solutions from a fresh kit and confirm that the correct volume of Cell Lysis Buffer is added.
	50% ethanol may have been prepared incorrectly. Confirm that you are using 95–100% USP/ACS, or molecular biology-grade ethanol diluted with molecular biology-grade water.
	Make sure that samples are thoroughly mixed and the resin is disrupted during the tip mixing in Wash #1. Incomplete disruption of the resin during this stage can affect eluate purities.
	Confirm that you are blanking your spectrophotometer with the water used to elute the samples or with the appropriate concentration of TE used to store the samples. Blanking with other solutions can lead to aberrant readings.
	Severely compromised blood can sometimes cause purity ratios below the expected range. If you are concerned about purity of DNA, test with fresh blood to verify that the chemistry is performing appropriately.

Symptoms	Possible Causes and Comments
Eluated DNA is discolored	Incomplete lysis of blood cells. Failure to add sufficient volume of Proteinase K Solution, Alkaline Protease or Cell Lysis Buffer will result in incomplete lysis and digestion of cells and proteins.
	ReliaPrep™ Resin in final eluate. Concentrated DNA solutions can be viscous. Additional time may be required to capture the ReliaPrep™ Resin from viscous solutions. Small amounts of resin may be transferred during manual purification. The eluted DNA should be collected slowly and any residual resin removed from the eluated DNA by centrifugation.
Degraded DNA	Nucleases were introduced during purification and handling. Use nuclease-free plasticware or glassware. Use aerosol-resistant tips during all pipetting steps. Wear gloves at all times. Nucleases introduced after elution will degrade DNA. Add 10X or 20X 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 also will be degraded. Repeat DNA purification with new starting material. Be sure that starting material has been handled to minimized DNA degradation.

7. References

1. Wilfinger, W., Mackey, K. and Chomczynski, P. (1997) Effect of pH and ionic strength on the spectrophotometric assessment of nucleic acid purity. *BioTechniques* **22**, 474–81.
2. Glasel, J.A. (1995) Validity of nucleic acid purities monitored by 260nm/280nm absorbance ratios. *BioTechniques* **18**, 62–3.
3. Manchester, K.L. (1995) Value of A_{260}/A_{280} ratios for measurement of purity of nucleic acids. *BioTechniques* **19**, 208–10.

8. Related Products

Product	Size	Cat.#
HSM 2.0 Instrument	1 each	A2715
HSM 2.0 Tube Rack	1 each	A2713
HSM 2.0 Tube Rack Stand	1 each	A2714
2.2ml Square-Well Deep Well Plate	50/case	V6781
RNase A Solution	5ml	A7974
20X TE Buffer (pH 7.5)	25ml	A2651
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	each	A2691

9. Summary of Changes

The following changes were made to the 11/18 revision of this document:

1. On p. 2, the range of A_{260}/A_{230} ratio was changed from "between 1.8 and 2.2" to "between 1.5 and 2.4" per a change in resin.
2. Section 7. Troubleshooting was updated to add possible causes and solutions for cases of low DNA purity.

[a]U.S. Pat. No. 6,855,499, European Pat. Nos. 1368629, 2090655 and 2363476, Japanese Pat. No. 4399164 and other patents.