Technical Manual

ReliaPrep™ 96 gDNA Miniprep HT System

INSTRUCTIONS FOR USE OF PRODUCTS A2670 AND A2671.

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ReliaPrep™ 96 gDNA Miniprep HT System

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1. Description

The ReliaPrep™ 96 gDNA Miniprep HT System[a] provides a simple and reliable method for the rapid isolation of genomic DNA (gDNA) in a multiwell format. Because the system is robust, gDNA may be purified from anti-coagulated whole blood or saliva collected in Oragene®•Discover devices. The purified gDNA can be used directly in PCR assays, microarrays, and next-generation sequencing applications. The use of paramagnetic particles for DNA capture eliminates the need for centrifugation or vacuum manifolds, making the system suitable for full automation. In addition, the system does not require phenol or chloroform, making it safe and convenient.

The ReliaPrep™ 96 gDNA Miniprep HT System uses Proteinase K and a specially formulated Cell Lysis Buffer to liberate sample DNA. (Note: The Cell Lysis Buffer is only required for blood samples.) Released DNA is bound to the ReliaPrep™ particles in the presence of the Binding Buffer. The DNA bound to Resin is captured by a magnet, and contaminants are removed by washing with the Wash Buffer and ethanol. The DNA is then eluted from the particles with 25mM Tris-HCl. The process involves the following simple steps:

- cell lysis with Proteinase K and Lysis Buffer (for blood samples only)
- gDNA capture to paramagnetic resin in Binding Buffer
- washes with Wash Buffer and ethanol
- elution with Tris-HCl

2. Product Components and Storage Conditions

Product	Size	Cat.#
ReliaPrep™ 96 gDNA Miniprep HT System	1 × 96	A2670

For in vitro Research Use Only. Sufficient reagents to process one 96-well plate of samples. Includes:

- 5.5ml Proteinase K (PK) Solution
- 40ml Cell Lysis Buffer (CLD)
- 5.5ml ReliaPrep™ Resin
- 50ml Binding Buffer (BBA)
- 85ml Wash Buffer (WBA)
- 60ml 25mM Tris-HCl (pH 8.0)
- 10ml 10mM EDTA (pH 8.0)
- 1ml RNase A Solution, 4mg/ml

Product	Size	Cat.#
ReliaPrep™ 96 gDNA Miniprep HT System	4 × 96	A2671

For in vitro Research Use Only. Sufficient reagents to process four 96-well plates of samples. Includes:

- 23ml Proteinase K (PK) Solution
- 160ml Cell Lysis Buffer (CLD)
- 4 × 5.5ml ReliaPrep™ Resin
- 200ml Binding Buffer (BBA)
- 350ml Wash Buffer (WBA)
- 60ml 25mM Tris-HCl (pH 8.0)
- 10ml 10mM EDTA (pH 8.0)
- 5ml RNase A Solution, 4mg/ml

Storage Conditions: Store all ReliaPrep™ 96 gDNA Miniprep HT System components at 15–30°C.

Items Available Separately

Product	Size	Cat.#
Heat Block Adapter	1 each	A2661
RNase A	5ml	A7974
25mM Tris-HCl (pH 8.0)	60ml	A2641
10mM EDTA (pH 8.0)	10ml	A2631
Deep Well MagnaBot® 96 Magnetic Separation Device	1 each	V3031

Note: Throughout the remainder of this document, the supplied Cell Lysis Buffer (CLD), Binding Buffer (BBA) and Wash Buffer (WBA) are referred to as Cell LysisBuffer, Binding Buffer and Wash Buffer, respectively.

3. System Requirements

This protocol requires the Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031). This protocol has been optimized using the V&P Scientific Heating Block (Cat.# V6761) with a Heat Block Adapter (Cat.# A2661) designed for a 2.2ml deep-well 96-well plate (Cat. # V6781).

Materials to Be Supplied by the User

- Heat Block Adapter (Cat.# A2661)
- heat block (e.g., V&P Scientific Heating Block; Cat.# V6761)
- Deep Well MagnaBot® 96 Magnetic Separation Device (Cat.# V3031)
- 3 × 2.2ml deep-well, 96-well plates (one for samples, one for lysis waste, one for wash waste; Cat. # V6781)
- elution plate
- 47.5–50% ethanol (Note: Prepare by diluting 95–100% USP/ACS- or molecular biology-grade ethanol with an equal volume of molecular biology grade water. Using denatured ethanol that contains methanol or isopropanol may decrease DNA purity or yield.)

Note: Use of labware different than listed above is not recommended.

4. Description of the Automated ReliaPrep™ 96 gDNA Miniprep HT System Method

4.A. Purification of Genomic DNA from Anticoagulated Whole Blood

This overview describes the general liquid-handling steps required for the automated ReliaPrep™ 96 gDNA Miniprep HT System method.

1. **Sample Preparation.** The following protocol describes the chemistry for purifying gDNA from 350µl of input sample. The sample must be dispensed into a 2.2ml deep-well plate. Volumes of anticoagulated whole blood from 100–350µl can be processed with this chemistry.

   Note: The heat block temperature must be set to 85°C before starting the method and should remain at that temperature for the duration of the method. Using a heat block temperature other than 85°C will result in suboptimal performance of the chemistry.

2. **Proteinase K Addition.** The liquid-handling robot adds 35µl of Proteinase K to each sample in the sample plate.

3. **Lysis Buffer Addition.** The liquid-handling robot adds 350µl of Cell Lysis Buffer to each sample in the sample plate.

4. **Lysis Incubation.** The samples are alternately incubated and mixed: 10-minute incubation, 30 seconds shaking and 10-minute incubation.

4.A. Purification of gDNA from Anticoagulated Whole Blood (continued)

5. **Binding Buffer Addition.** The liquid-handling robot adds 420µl of Binding Buffer to each sample in the sample plate.

   Note: If RNase treatment is desired, prepare a 0.1mg/ml solution of RNase A (Cat.# A7974) in Binding Buffer when preparing and dispensing reagents. Add RNase A to the reservoir before adding Binding Buffer. Mix the RNase A and Binding Buffer by pipetting using a serological pipette.

6. **Resin Addition.** The liquid-handling robot then adds 35µl of ReliaPrep™ Resin to each sample in the sample plate.

7. **DNA Binding.** The sample plate is incubated for 20 minutes with shaking.

8. **Cleared Lysate Removal.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device and incubated for 5 minutes to capture magnetic resin. The liquid-handling robot removes the cleared lysate to the lysate waste plate.

9. **Wash Buffer Addition #1.** The liquid-handling robot adds 400µl of Wash Buffer and 50µl of 47.5–50% ethanol to each sample in the sample plate. The samples are mixed in a series of orbital shaking and pipetting steps:

   a. The plate is mixed for 45 seconds by orbital shaking.

   b. The material in the sample wells is mixed 8 times by pipetting 125µl.

   c. The plate is mixed for 45 seconds by orbital shaking.

   d. The material in the sample wells is mixed 8 times by pipetting 125µl.

   e. The plate is mixed for 30 seconds by orbital shaking.

10. **Wash Buffer Removal #1.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device to capture magnetic resin for 120 seconds. The liquid-handling robot removes the Wash Buffer waste to the wash waste plate.

11. **Wash Buffer Addition #2.** The liquid-handling robot adds 400µl of Wash Buffer and 50µl of 47.5–50% ethanol to each sample in the sample plate. The samples are mixed in a series of orbital shaking and pipetting steps as in step 9.

12. **Wash Buffer Removal #2.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device for 120 seconds to capture magnetic resin. The liquid-handling robot removes the Wash Buffer waste to the wash waste plate.

13. **Ethanol Wash Addition.** The liquid-handling robot adds 450µl of ethanol to each sample in the sample plate. The samples are mixed in a series of orbital shaking and pipetting steps:

    a. The plate is mixed for 45 seconds by orbital shaking.

    b. The material in the sample wells is mixed 12 times by pipetting 125µl.

    c. The plate is mixed for 30 seconds by orbital shaking.

14. **Ethanol Wash Removal.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device to capture magnetic resin for 120 seconds. The liquid-handling robot removes the ethanol wash waste to the wash waste plate.

15. **Heat Block Drying.** The sample plate is moved onto the heat block for 45 seconds and then returned to the Deep Well MagnaBot® 96 Magnetic Separation Device for 5 minutes.

16. **Tris Buffer Addition.** The liquid-handling robot adds 110µl of 25mM Tris-HCl (pH 8.0) to each sample in the sample plate. The samples undergo a series of shaking and incubations (30-second shaking, 3-minute incubation, 3-minute shaking, 30-minute incubation and 30-second shaking) to elute DNA from the ReliaPrep™ Resin into the Tris Buffer.

17. **Elution.** The Sample Plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device, magnetic resin is captured for 2 minutes, and the supernatant is removed to the elution plate.

18. **Method Ends.** The automated ReliaPrep™ 96 gDNA Miniprep HT System method is now complete. The purified DNA samples in the elution plate may be processed immediately or stored at 4°C. For prolonged stability add 10µl of the 10mM EDTA (pH 8.0) included with the ReliaPrep™ 96 gDNA Miniprep HTS Purification System. The EDTA should only be added after completion of absorbance readings.

4.B. Purification of gDNA from Saliva in Oragene®•Discover Devices

This overview describes the general liquid-handling steps required for the automated ReliaPrep™ 96 gDNA Miniprep HT System method.

1. **Sample Preparation.** The following protocol describes the chemistry for purifying gDNA from 700µl of input sample. The saliva collected in Oragene®•Discover device must be dispensed into a 2.2ml deep-well plate.

   Note: The heat block temperature must be set to 70°C before starting and should remain at that temperature for the duration of the method. Using a heat block temperature other than 70°C will result in suboptimal performance of the chemistry.

2. **Mix.** The samples are mixed by orbital shaking for 60 seconds.

3. **Binding Buffer Addition.** The liquid-handling robot adds 420µl of Binding Buffer to each sample in the sample plate.

   Note: If RNase treatment is desired, prepare a 0.1mg/ml solution of RNase A (Cat.# A7974) in Binding Buffer. Add RNase A to the reservoir before adding Binding Buffer. Mix the RNase A and Binding Buffer by pipetting using a serological pipette.

4. **Resin Addition.** The liquid-handling robot then adds 35µl of ReliaPrep™ Resin to each sample in the sample plate.

5. **DNA Binding.** The sample plate is incubated for 20 minutes with shaking.

6. **Cleared Lysate Removal.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device and incubated for 5 minutes to capture magnetic resin. The liquid-handling robot removes the cleared lysate to the lysate waste plate.

7. **Wash Buffer Addition #1.** The liquid-handling robot adds 265µl of Wash Buffer and 35µl of 47.5–50% ethanol to each sample in the sample plate. The samples are mixed in a series of orbital shaking and pipetting steps:

   a. The plate is mixed for 45 seconds by orbital shaking.

   b. The material in the sample wells is mixed 8 times by pipetting 125µl.

   c. The plate is mixed for 45 seconds by orbital shaking.

   d. The material in the sample wells is mixed 8 times by pipetting 125µl.

   e. The plate is mixed for 30 seconds by orbital shaking.

8. **Wash Buffer Removal #1.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device to capture magnetic resin for 120 seconds. The liquid-handling robot removes the Wash Buffer waste to the wash waste plate.

9. **Wash Buffer Addition #2.** The liquid-handling robot adds 265µl of Wash Buffer and 35µl of 47.5–50% ethanol to each sample in the sample plate. The sample plate is mixed for 60 seconds by orbital shaking.

10. **Wash Buffer Removal #2.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device for 120 seconds to capture magnetic resin. The liquid-handling robot removes the Wash Buffer waste to the wash waste plate.

11. **Wash Buffer Addition #3.** The liquid-handling robot adds 265µl of Wash Buffer and 35µl 47.5–50% ethanol to each sample in the sample plate. The sample plate is mixed for 60 seconds by orbital shaking.

12. **Wash Buffer Removal #3**. The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device to capture magnetic resin for 120 seconds. The liquid-handling robot removes the Wash Buffer to the wash waste plate.

13. **Ethanol Wash Addition.** The liquid-handling robot adds 450µl of ethanol to each sample in the sample plate. The samples are mixed for 120 seconds by orbital shaking.

14. **Ethanol Wash Removal.** The sample plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device to capture magnetic resin for 120 seconds. The liquid-handling robot removes the ethanol wash waste to the wash waste plate.

15. **Heat Block Drying.** The sample plate is moved onto the heat block for 45 seconds and then returned to the Deep Well MagnaBot® 96 Magnetic Separation Device for 5 minutes.

16. **Tris Buffer Addition.** The liquid-handling robot adds 110µl of 25mM Tris-HCl (pH 8.0) to each sample in the sample plate. The samples undergo a series of shaking and incubations (30-second shaking, 3-minute incubation, 3-minute shaking, 30-minute incubation and 30-second shaking) to elute DNA from the ReliaPrep™ Resin into the Tris Buffer.

17. **Elution.** The Sample Plate is moved onto the Deep Well MagnaBot® 96 Magnetic Separation Device, magnetic resin is captured for 2 minutes, and the supernatant is removed to the elution plate.

18. **Method Ends.** The automated ReliaPrep™ 96 gDNA Miniprep HT System method is complete. The purified DNA samples in the elution plate may be processed immediately or stored at 4°C. For prolonged stability add 10µl of the 10mM EDTA (pH 8.0) included with the ReliaPrep™ 96 gDNA Miniprep HTS Purification System. The EDTA should only be added after completion of absorbance readings.

5. Quantitation and Analysis of Isolated Genomic DNA

Concentration and purity of eluted DNA can be analyzed directly using a spectrophotometer. The molecular weight of eluted DNA can be analyzed by agarose gel electrophoresis.

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. Consult your spectrophotometer user manual to determine whether this calculation is performed.

Other techniques such as gel analysis also may be valuable when assessing the relative quality of isolated genomic DNA. We consistently observe relatively poor agreement between concentration values obtained from the same sample using different quantitation methodologies. This has been observed using a variety of commercially available purification systems, and therefore, we do not recommend comparing yields obtained using different quantitation methods. In our experience, spectrophotometric analysis coupled with functional testing in downstream applications is typically the most representative assessment of sample quantity and quality.

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	Insufficient mixing of sample during automated method. Vigorous mixing during lysis, binding and elution is essential to ensure efficient capture of DNA from the sample. Check both tip mixing and orbital shaking to verify that they are effective.
	Insufficient resuspension of ReliaPrep™ Resin before dispensing to the reservoir on the robot deck. Thoroughly mix the ReliaPrep™ Resin reagent bottle, this includes inverting the bottle to confirm that no resin adheres to the bottle.
	Sample contained too few white cells per milliliter of blood. DNA yield depends on the amount of starting material. Blood samples with low white cell counts will have reduced yields because of the low sample input.
	Insufficient elution volume. If the volume of 25mM Tris-HCl (pH 8.0) is reduced to try to improve eluate concentration, it may result in lower yield.
	Ensure reagents are in the correct deck locations. 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.
Eluted DNA looks brown or red	Incomplete lysis of blood cells. Failure to add sufficient volume of Proteinase K or Cell Lysis Buffer will result in incomplete lysis and digestion of cells and proteins.
Degraded DNA	Nucleases were introduced during purification. 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. Add EDTA to a final concentration of 0.1–1mM to protect eluted samples from nucleases.

6. Troubleshooting (continued)

Symptoms	Causes and Comments
Degraded DNA (continued)	Old samples or samples that have been handled or stored improperly may contain DNA that is already degraded.
	DNA was eluted with water instead of TE.
ReliaPrep™ Resin in final eluate	Concentrated DNA solutions can be viscous. Additional time may be required to capture ReliaPrep™ Resin from such viscous solutions. The eluted DNA should be collected slowly and any residual resin removed from the eluted DNA by performing additional magnetic captures or a centrifugation.

7. References

1. Wilfinger, W.W., Mackey, M. and Chanczynski, 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

High-Throughput Genomic DNA Isolation

Product	Size	Cat.#
ReliaPrep™ Large Volume HT gDNA Isolation System	96 × 10ml preps	A1751
ReliaPrep™ LV 32 HSM Instrument	1 each	A1715

Low-Throughput Nucleic Acid Isolation

Product	Size	Cat.#
ReliaPrep™ gDNA Tissue Miniprep System	250 preps	A2052
ReliaPrep™ gDNA Blood Miniprep System	250 preps	A5082
ReliaPrep™ FFPE Total RNA Miniprep System	100 reactions	Z1002
ReliaPrep™ FFPE gDNA Miniprep System	100 reactions	A2352

Additional sizes available.

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

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Product claims are subject to change. Please contact Promega Technical Services or access the Promega online catalog for the most up-to-date information on Promega products.

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