Technical Manual

ReliaPrep™ Blood gDNA Miniprep System

INSTRUCTIONS FOR USE OF PRODUCTS A5080, A5081 AND A5082.

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Part# TM330

ReliaPrep™ Blood gDNA Miniprep System

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

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1. Description

The ReliaPrep™ Blood gDNA Miniprep System[a] provides a fast, simple technique for preparation of purified and intact DNA from mammalian blood. Samples are processed using a binding column in a microcentrifuge tube. Up to 200µl of blood can be processed per purification. The genomic DNA isolated is of high-quality and can be used in common applications such as agarose gel analysis, restriction enzyme digestion and PCR analysis.

The ReliaPrep™ Blood gDNA Miniprep System uses a simple four-step method:

1. Effectively disrupting or homogenizing the starting material to release the DNA.
2. Binding DNA to the ReliaPrep™ Binding Column.
3. Removing impurities with wash solution.
4. Eluting purified DNA.

No ethanol is used in the purification protocol, eliminating downstream problems caused by ethanol carryover.

2. Product Components and Storage Conditions

Product	Size	Cat.#
ReliaPrep™ Blood gDNA Miniprep System—Sample Size	10 preps	A5080

Each system contains sufficient reagents for 10 purifications. Includes:

- 1 pack ReliaPrep™ Binding Columns (10/pack)
- 1 pack Collection Tubes (40/pack)
- 2.5ml Cell Lysis Buffer (CLD)
- 250µl Proteinase K (PK) Solution
- 3ml Binding Buffer (BBA)
- 17ml Column Wash Solution (CWD)
- 13ml Nuclease-Free Water

Product	Size	Cat.#
ReliaPrep™ Blood gDNA Miniprep System	100 preps	A5081

Each system contains sufficient reagents for 100 purifications. Includes:

- 2 packs ReliaPrep™ Binding Columns (50/pack)
- 10 packs Collection Tubes (40/pack)
- 22ml Cell Lysis Buffer (CLD)
- 1.1ml Proteinase K (PK) Solution
- 27.5ml Binding Buffer (BBA)
- 165ml Column Wash Solution (CWD)
- 50ml Nuclease-Free Water

Product	Size	Cat.#
ReliaPrep™ Blood gDNA Miniprep System	250 preps	A5082

Each system contains sufficient reagents for 250 purifications. Includes:

- 5 packs ReliaPrep™ Binding Columns (50/pack)
- 5 packs Collection Tubes (200/pack)
- 55ml Cell Lysis Buffer (CLD)
- 5.5ml Proteinase K (PK) Solution
- 68.75ml Binding Buffer (BBA)
- 412.5ml Column Wash Solution (CWD)
- 50ml Nuclease-Free Water

Storage Conditions: All components should be stored at 15–30°C.

3. Protocol

Materials to Be Supplied by the User

- rotisserie mixer for resuspension of whole blood
- vortex mixer
- 1.5ml microcentrifuge tubes
- heating block set to 56°C
- microcentrifuge capable of 14,000 × *g*

1. Thoroughly mix the blood sample for at least 10 minutes in a rotisserie shaker at room temperature. If the blood has been frozen, thaw completely before mixing for 10 minutes.
2. Dispense 20µl of Proteinase K (PK) Solution into a 1.5ml microcentrifuge tube.
3. Add 200µl of blood to the tube containing the Proteinase K (PK) Solution, and briefly mix.
4. Add 200µl of Cell Lysis Buffer (CLD) to the tube. Cap and mix by vortexing for at least 10 seconds.

(!) This vortexing step is essential for obtaining good yields.

5. Incubate at 56°C for 10 minutes.
6. While the blood sample is incubating, place a ReliaPrep™ Binding Column into an empty Collection Tube.
7. Remove the tube from the heating block. Add 250µl of Binding Buffer (BBA), cap the tube, and mix by vortexing for 10 seconds with a vortex mixer.

   Note: The lysate should be dark green at this point.

(!) This vortexing step is essential for obtaining good yields.

8. Add the contents of the tube to the ReliaPrep™ Binding Column, cap it and place it in a microcentrifuge.
9. Centrifuge for 1 minute at maximum speed. Check the binding column to make sure the lysate has completely passed through the membrane. If lysate is still visible on top of the membrane, centrifuge the column for another minute.

   Note: The sample can be centrifuged at lower speed, if desired. Increase the centrifugation time accordingly to ensure the lysate has completely passed through the membrane.
10. Remove the collection tube containing flowthrough, and discard the liquid as hazardous waste.
11. Place the binding column into a fresh collection tube. Add 500µl of Column Wash Solution (CWD) to the column, and centrifuge for 3 minutes at maximum speed. Discard the flowthrough.

    Note: If any of the wash solution remains on the membrane, centrifuge the column for another minute.

3. Protocol (continued)

12. Repeat Step 11 twice for a total of three washes.

13. Place the column in a clean 1.5ml microcentrifuge tube.

14. Add 50–200µl of Nuclease-Free Water to the column. Centrifuge for 1 minute at maximum speed.

    Note: Eluting in 50µl significantly increases the concentration of the DNA but reduces yield by 25–30%.

15. Discard the ReliaPrep™ Binding Column, and save eluate. Do not reuse binding columns or collection tubes.

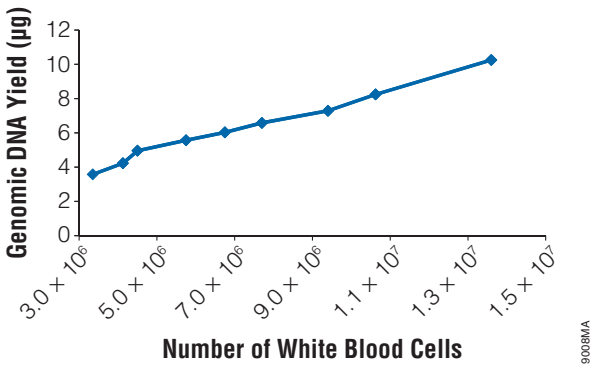

Figure 1. The yield of genomic DNA varies with white blood cell count. Whole blood was obtained from several individuals, and white cell counts were determined using a hemocytometer. Two hundred microliters of blood was used for genomic DNA purification (n = 3 or 4), and the amount of isolated gDNA was quantitated by absorbance spectroscopy.

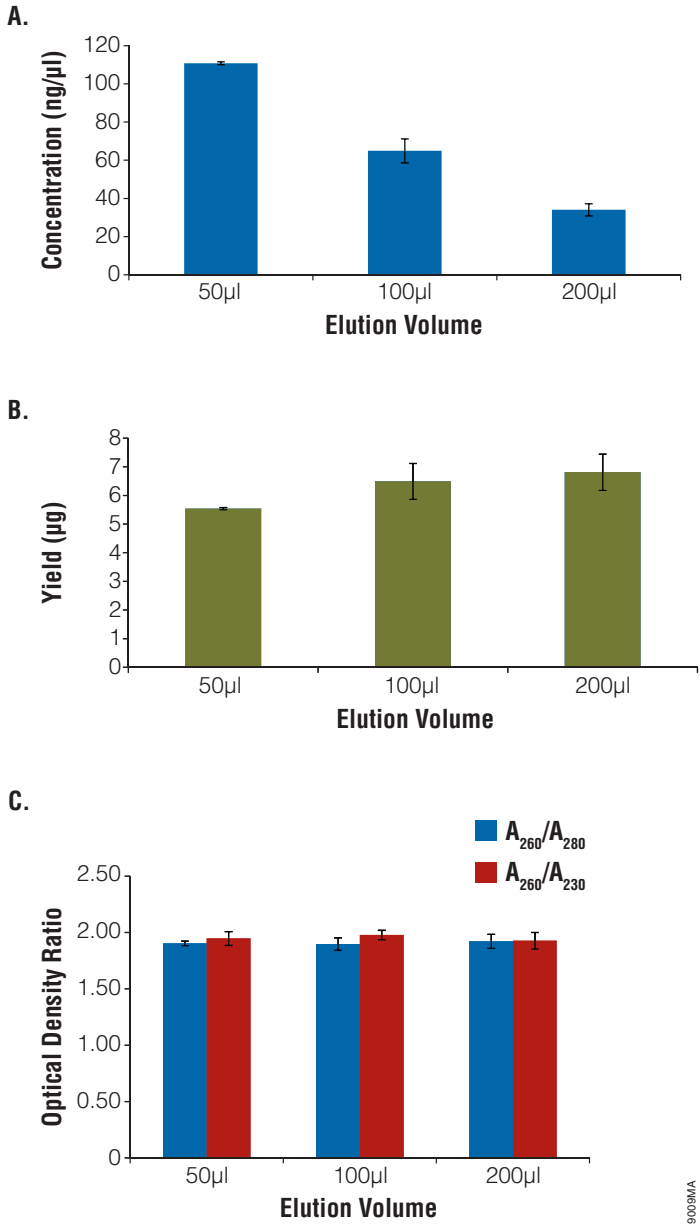

Figure 2. Comparison of elution volume with concentration, yield and purity. Aliquots of blood (200µl) were processed using the ReliaPrep™ Blood gDNA Minprep System (n = 4) and eluted with 30–200µl of Nuclease-Free Water. Concentration (**Panel A**), total yield (**Panel B**) and purity (**Panel C**) were assessed using absorbance spectroscopy. Yield decreased slightly with decreases in elution volume, while concentration increased. Purity as measured by optical density ratios remained constant.

4. 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
Blood forms clots and lysate did not pass through the column	Blood was not sufficiently mixed. For good lysis, the blood must be mixed prior to adding proteinase K and lysis buffer.
	Sample not vortexed after adding lysis buffer. Mixing is required for optimal results
Wash buffer did not pass through the column	Samples were not centrifuged long enough. Recentrifuge for 1 minute.
	Centrifuge was not generating sufficient *g* force. The ReliaPrep™ Blood gDNA Miniprep System and columns are designed for use with a microcentrifuge capable of generating at least 12,000 × *g*. Small microcentrifuges designed for capturing materials in tubes may not generate sufficient force to pass the column wash buffer through the column matrix.
DNA yield was low	Blood contained low levels of leukocytes. Check the level of white blood cells using a hemocytometer. White blood cell levels less than 4×10^6 per milliliter will give reduced yields.
	Blood was not properly resuspended. Make sure that the blood is resuspended for at least 10 minutes in a rotary shaker. Vigorous vortexing also can be used to resuspend blood.
	Blood was too old. Best yields are obtained with fresh blood. Samples that have been stored at 2–5°C for more than 5 days may give reduced yields.
	Lysis was incomplete. Make sure to vortex for a minimum of 10 seconds after adding lysis buffer to the blood. After the heating step, the blood/lysis buffer mixture should be a dark green color. If it is not, lysis was incomplete.
	Binding solution was not mixed properly with lysate. Make sure to vortex the solution for at least 10 seconds after adding the binding buffer to the heated lysate.

Symptoms	Causes and Comments
Degraded DNA	Improper collection or storage of blood. Obtain a new sample under the proper conditions.
	Avoid multiple freeze-thaw cycles of purified DNA.

[a]U.S. Pat. No. 7,264,927, European Pat. No. 1442045, Japanese Pat. No. 4277115, Australian Pat. No. 2002333673 and other patents pending.

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All prices and specifications are subject to change without prior notice.

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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