Isolating gDNA from Packed Cell Pellets

Simplified gDNA Isolation from Human Blood Packed Cell Pellets Using the ReliaPrep™ Large Volume HT gDNA Isolation System

Promega Corporation

Sample Type:
Packed red cell pellets or packed all cell pellets prepared from human blood, collected in Vacutainer® Tubes with common anti-coagulants (EDTA, citrate and heparin)

Sample Volume:
2–10ml (original whole blood volume used to prepare packed cell pellets)

Yield: 150–300µg from 10ml samples containing from 5×10^6 to 1×10^7 WBC/ml. Yield depends on the white blood cell (WBC) count of the sample.

Purity: $A_{260}/A_{280} > 1.7$

$A_{260}/A_{230} = 1.8–2.2$

Size: Greater than 25kb

Eluted Samples:
Ready for downstream assays/ archiving

Protocol: *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual #*TM341

The ReliaPrep™ Large Volume HT gDNA Isolation System is a scalable, automation-ready system that simplifies gDNA isolation from human blood.

Introduction

Packed blood cell pellets are created by centrifugation of a whole blood sample and removal of the plasma fraction (packed all cell pellet) or the plasma and white blood cell fractions (packed red cell pellet). Processing with the ReliaPrep™ Large Volume HT gDNA Isolation system is based on using a whole blood sample starting volume of no more than 10ml. Packed blood cell pellet samples are placed into the ReliaPrep™ LV 32 HSM Instrument in 50ml conical tubes for processing. For semi-automated processing, the ReliaPrep™ LV 32 HSM Instrument guides the user through reagent additions and aspirations via its LCD screen based on the original whole blood sample volume used to create the packed blood cell pellet. For automated processing, the liquid handler will perform processing steps, scaling the reagent additions for each sample based on the sample volumes detected. The volume of the packed cell pellet is doubled to estimate the original whole blood volume used to create the packed cell pellet sample. Samples are processed based on this estimated original sample volume, with all samples below 3ml estimated original blood volume being processed through a low-volume fixed protocol, all samples with an estimated original blood volume of 3–10ml being processed with a scaled protocol, and all estimated volumes over 10ml being processed with a high-volume fixed protocol.

Protocol for Less Than 1.5ml Packed Cell Pellet Volume

1. Proteinase K Solution (60µl) is added to each sample.
1. Optional: RNase (60µl) is added to each sample.
2. Alkaline Protease (375µl) is added to each sample.
3. Three milliliters of Lysis Buffer is added to each sample.
4. After Lysis Buffer is added, samples are incubated at 65°C for 20 minutes with shaking at 500rpm, followed by 20 minutes of shaking at 500rpm without heat.
5. Binding Buffer (3.6ml) is added to each sample.
6. ReliaPrep™ Resin is thoroughly resuspended, and 300µl of resin is added to each sample. Nucleic acids bind to the resin during a 20-minute room-temperature incubation at 500rpm. The resin is collected for 14 minutes using a magnet.
7. Waste from the lysis and binding steps is removed from each tube. After waste removal, 1ml of Prepared Wash Buffer is added to that tube. This step is repeated until all tubes have had waste removed and wash added.

8. Samples are shaken at 600rpm for 2 minutes.
9. After shaking, samples are tip-mixed to thoroughly disperse the resin. The instrument adds an additional 4.4ml of Prepared Wash Buffer and shakes at 600rpm for 3 more minutes. This is followed by 3 minutes of magnetic capture.
10. Waste from the first wash step is removed from each tube. After waste removal, 1ml of Prepared Wash Buffer is added to that tube. This step is repeated until all tubes have had waste removed and wash added. After all waste has been removed, an additional 4.4ml of Prepared Wash Buffer is added to the samples while shaking. Samples are shaken at 600rpm for 4 minutes followed by magnetic capture for 3 minutes.
11. Waste from the second wash step is removed from each tube. After waste removal, 4.4ml of Ethanol Wash is added to that tube. This step is repeated until all tubes have had waste removed and wash added. Samples are shaken at 600rpm for 4 minutes followed by magnetic capture for 3 minutes.
12. All waste is removed by column, and Nuclease-Free Water is added to each tube. Samples are shaken at 600rpm for 3 minutes, and then at 400rpm for 15 minutes at 80°C. Magnetic capture is performed for 4 minutes, and the eluates are transferred to the intermediate plate.
13. The user is prompted to centrifuge the intermediate plate at 2,500 × *g* for 10 minutes to remove any particulates.
14. The intermediate plate is placed back on the instrument, and the eluates are transferred to the final elution labware.
15. The method is finished.

Protocol for 1.5–5ml Packed Cell Pellet Volume

1. Proteinase K Working Solution (0.02 volumes based on estimated original blood sample volume) is added to each tube.
2. Optional: RNase (0.02 volumes based on estimated original blood sample volume) is added to each sample.
3. Alkaline Protease (0.125 volumes based on estimated original blood sample volume) is added to each sample.
4. One volume of Lysis Buffer based on estimated original blood sample volume is added to each sample.
5. After Lysis Buffer is added, samples are incubated at 65°C for 20 minutes with shaking at 500rpm, followed by 20 minutes of shaking at 500rpm without heat. Next, 1.2 volumes of Binding Buffer based on estimated original blood sample volume is added to each sample.
6. ReliaPrep™ Resin is thoroughly resuspended, and 0.1 volumes of resin based on estimated original blood sample volume is added to each sample. Binding of nucleic acid to the resin is accomplished through incubation at room temperature for 20 minutes at 500rpm followed by magnetic capture for 14 minutes to collect the resin.
7. Waste from lysis and binding is removed from each tube. After waste removal, 1–3ml of Prepared Wash Buffer based on estimated original blood sample volume is added to that tube. This step is repeated until all tubes have had waste removed and wash added.
8. Samples are shaken at 600rpm for 2 minutes.
9. After shaking, the samples are tip-mixed to thoroughly disperse the resin. Following this, the instrument adds additional Prepared Wash Buffer in the range of 4.4–9ml based on estimated original blood sample volume and shakes at 600rpm for 3 more minutes. This is followed by 3 minutes of magnetic capture.
10. Waste from the first wash step is removed from each tube. Then 1ml of Prepared Wash Buffer is added to the samples. While shaking, the instrument adds additional Prepared Wash Buffer in the range of 4.4–9ml based on estimated original blood sample volume and shakes at 600rpm for 4 more minutes, followed by 3 minutes of magnetic capture.
11. Waste from the second wash step is removed from each tube. Then 4.4–9ml of Ethanol Wash is added to the samples that are shaken for 4 minutes at 600rpm. The samples are then subjected to magnetic capture for 3 minutes.
12. All waste is removed by column, and the calculated amount of Nuclease-Free Water is added to each tube. Samples are shaken at 600rpm for 3 minutes, and then at 400rpm for 15 minutes at 80°C. Magnetic capture is performed for 4 minutes, and the eluates are transferred to the intermediate plate.
13. The user is prompted to centrifuge the intermediate plate at 2,500 × *g* for 10 minutes to remove any particulates.
14. The intermediate plate is placed back on the instrument, and the eluates are transferred to the final elution labware.
15. The method is finished.

Protocol for Greater Than 5ml Packed Cell Pellet Volume
(A maximum of 10ml of whole blood can be used to create packed cell pellet samples.)

1. Proteinase K Solution (600µl) is added to each sample.
2. Optional: RNase (600µl) is added to each sample.
3. Alkaline Protease (1,250µl) is added to each sample.
4. Ten milliliters of Lysis Buffer is added to each sample.
5. After Lysis Buffer is added, samples are incubated at 65°C for 20 minutes with shaking at 500rpm, followed by 20 minutes of shaking at 500rpm without heat.
6. Binding Buffer (12ml) is added to each sample.
7. ReliaPrep™ Resin is thoroughly resuspended, and 1,000µl of resin is added to each sample. Nucleic acids bind to the resin during a 20-minute room-temperature incubation at 500rpm. The resin is collected for 14 minutes using a magnet.
8. Waste from lysis and binding is removed from each tube. After waste removal, 3ml of Prepared Wash Buffer is added to that tube. This step is repeated until all tubes have had waste removed and wash added.
9. Samples are shaken at 600rpm for 2 minutes.
10. After shaking, the samples are tip-mixed to thoroughly disperse the resin. Following this, the instrument adds 9ml of additional Prepared Wash Buffer and shakes at 600rpm for 3 more minutes. This is followed by 3 minutes of magnetic capture.
11. Waste from the first wash step is removed from each tube. After waste removal, 1ml of Prepared Wash Buffer is added to that tube. This step is repeated until all tubes have had waste removed and wash added. After all waste has been removed, an additional 9ml of Prepared Wash Buffer is added to the sample while shaking. Samples are shaken at 600rpm for 4 minutes, followed by magnetic capture for 3 minutes.
12. Waste from the second wash step is removed from each tube. After waste removal, 9ml of Ethanol Wash is added to that tube. This step is repeated until all tubes have had waste removed and samples added. Samples are shaken at 600rpm for 4 minutes followed by magnetic capture for 3 minutes.
13. All waste is removed by column, and Nuclease-Free Water is added to each tube. Samples are shaken at 600rpm for 3 minutes, and then at 400rpm for 15 minutes at 80°C. Magnetic capture is performed for 4 minutes, and the eluates are transferred to the intermediate plate.
14. The user is prompted to centrifuge the intermediate plate at 2,500 × *g* for 10 minutes to remove any particulates.
15. The intermediate plate is placed back on the instrument, and the eluates are transferred to the final elution labware.
16. The method is finished.

Ordering Information

Product	Size	Cat.#
ReliaPrep™ Large Volume HT gDNA Isolation System*	96 × 10ml or 960 × 1ml preps	A1751
ReliaPrep™ LV 32 HSM Instrument	1 each	A1715

*For Laboratory Use.