Isolating gDNA from Blood

Simplified gDNA Isolation from Human Blood using the ReliaPrep™ Large Volume HT gDNA Isolation System

Promega Corporation

Sample Type:
Human Blood, collected in Vacutainer® Tubes with common anti-coagulants (EDTA, citrate, and heparin)

Sample volume:
1–10ml

Yield: 200–400µg 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

Samples are placed into the ReliaPrep™ LV 32 HSM Instrument in 50ml conical tubes for processing. For automated processing, the liquid handler performs processing steps, scaling reagent additions for each sample based on the sample volumes detected. 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 sample volume selected by the user. For the detailed semi-automated protocol, refer to the *ReliaPrep™ Large Volume HT gDNA Isolation System Technical Manual #*TM341. Samples with input volumes of 1–3ml are processed via a low-volume fixed protocol; larger sample volumes are processed with a scaled protocol.

Protocol for 1–3ml of Blood

1. The instrument heater is turned on to 50°C prior to addition of Proteinase K Solution.
2. Proteinase K Solution (60µl) is added to each tube.
3. Optional: RNase (60µl) is added to each sample.
4. Alkaline Protease (375µl) is added to each sample. Samples are incubated a total of 10 minutes.
5. Three milliliters of Lysis Buffer is added to each sample.
6. After Lysis Buffer is added, the samples are incubated at 75°C an additional 10 minutes with shaking at 500rpm, followed by 10 minutes of shaking at 500rpm without heat. Binding Buffer (3.6ml) is added to each sample.
7. ReliaPrep™ Resin is thoroughly resuspended, and 300µl of the resin is added to each sample. The 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 the lysis and binding steps is removed from each tube. After removal of waste, 1ml of Prepared Wash Buffer is added to each tube.
9. Samples are shaken at 600rpm for 2 minutes.
10. After shaking, the samples are tip-mixed to thoroughly disperse the resin, and the instrument adds 4.4ml 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 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 is removed, an additional 4.4ml of Prepared Wash Buffer is added to samples 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 removal of waste, 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 3 minutes of magnetic capture.
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.

Protocol for 3–10ml of Blood

1. The heater of the HSM Instrument is turned on to 50°C prior to addition of Proteinase K Solution.
2. Proteinase K Working Solution (0.02 volumes) is added to each tube.
3. Optional: RNase (0.02 volumes) is added to each sample.
4. Alkaline Protease (0.125 volumes) is added to each sample.
5. One volume of Lysis Buffer is added to each sample.
6. After Lysis Buffer is added, the samples are incubated at 75°C for an additional 10 minutes with shaking at 500rpm, followed by 10 minutes of shaking at 500rpm without heat. Next, 1.2 volumes of Binding Buffer is added to each sample.
7. ReliaPrep™ Resin is thoroughly resuspended, and 0.1 volumes of resin 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.
8. Waste from the lysis and binding is removed from each tube. After removal of waste, 1–3ml of Prepared Wash Buffer based on original blood sample volume are 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 additional Prepared Wash Buffer in the range of 4.4–9ml based on the original blood sample volume while shaking and shakes at 600rpm for 3 more minutes. Next, the resin is captured for 3 minutes.
11. Waste from the first wash is removed from each tube, and 1ml of Prepared Wash Buffer is added to the samples. Then the instrument adds additional Prepared Wash Buffer in the range of 4.4–9ml based on the original blood sample volume while shaking and shakes at 600rpm for 4 more minutes. Samples are then subjected to magnetic capture for 3 minutes.
12. Waste from the second wash is removed from each tube, and 4.4–9ml of Ethanol Wash is added to the samples based on the original blood sample volume. Then the instrument shakes at 600rpm for 4 minutes. The samples are then subjected to magnetic capture for 3 minutes.
13. 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, 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.