

# Isolating gDNA from Buffy Coat Samples

## Semi-Automated or Fully Automated Isolation of gDNA from Buffy Coat Samples Using the ReliaPrep™ Large Volume HT gDNA Isolation System

Promega Corporation

### Sample Type:

Buffy coat derived from human blood, collected in Vacutainer® Tubes with common anti-coagulants (EDTA, citrate, and heparin)

### Sample Volume:

0.5–1.5ml buffy coat fraction (corresponding to ~2.5–15ml blood)

### Yield:

50–400µg DNA  
Yield of gDNA will vary depending on input buffy coat volume, white blood cell count and preparation 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 allows semi-automated or fully automated processing of buffy coat samples.*

### Introduction

Semi-automated or fully automated processing of buffy coat samples prepared from 2.5–15ml of blood is possible with the ReliaPrep™ Large Volume gDNA Isolation System using the ReliaPrep™ LV 32 HSM Instrument. Up to 32 samples can be processed per instrument run. The isolated gDNA can be stored or used directly in common downstream applications.

### Manual Protocol

Manual processing of buffy coat samples can be achieved by running the 3ml ReliaPrep™ System protocol on the HSM instrument. The steps of the manual protocol are detailed below.

1. Optional: RNase (60µl) is added to each sample.
2. Proteinase K Solution (60µl) is added to each tube.
3. Alkaline Protease (375µl) is added to each sample.
4. Three milliliters of Lysis Buffer is added to each sample.
5. After Lysis Buffer is added, the 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 (3.6ml) is added to each sample.
7. ReliaPrep™ Resin is thoroughly resuspended, and 300µl of the 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 the lysis and binding steps is removed from each tube. After removal of waste, 1ml of Ethanol Wash and 4ml of Wash Buffer are added to each tube.
9. Samples are shaken at 600rpm for 2 minutes.
10. After shaking, the samples are tip-mixed 6 times to thoroughly disperse the resin, and the instrument shakes at 600rpm for 3 more minutes. This is followed by 3 minutes of magnetic capture.
11. All waste is removed from each tube, then 1ml of Ethanol Wash and 4ml of Wash Buffer are added to the samples. Samples are shaken at 550rpm for 4 minutes followed by magnetic capture for 3 minutes.

## ReliaPrep™ System gDNA Isolation

12. All waste is removed from each tube, and 4ml of Ethanol Wash is added to each tube. Samples are shaken at 550rpm for 4 minutes followed by 3 minutes of magnetic capture.
13. All waste is removed from each tube, and Nuclease-Free Water is added to each tube. Samples are shaken at 650rpm for 3 minutes, and then at 400rpm for 25 minutes at 80°C. Magnetic capture is performed for 5 minutes, and the eluates are transferred to intermediate labware.
14. The user should centrifuge the intermediate labware at  $2,500 \times g$  for 10 minutes to remove any particulates.
15. The eluates are transferred to the final elution labware.
16. The method is finished.

### Automated Protocol

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 the 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.
7. Binding Buffer (3.6ml) is added to each sample.
8. ReliaPrep™ Resin is thoroughly resuspended, and 300µl 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.
9. Waste from the lysis and binding is removed from each tube. After removal of waste, 1ml of Prepared Wash Buffer is added to that tube. This step is repeated until all tubes have had waste removed and wash added.
10. Samples are shaken at 600rpm for 2 minutes.
11. After shaking, the samples are tip-mixed to thoroughly disperse the resin. Then 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.
12. Waste from the first wash step is removed from each tube. After removal of waste, 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 and then subjected to magnetic capture for 3 minutes.
13. 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.
14. All waste is removed from each tube, and 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.
15. The user is prompted to centrifuge the intermediate plate at  $2,500 \times g$  for 10 minutes to remove any particulates.
16. The intermediate plate is placed back on the instrument, and the eluates are transferred to the final elution labware.
17. 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.

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