



## ReliaPrep™ Large Volume HT gDNA Isolation System: gDNA isolation from blood samples

The ReliaPrep™ Large Volume HT gDNA Isolation System enables automated purification of genomic DNA (gDNA) from up to 96 samples of whole blood at a time. The system dramatically increases laboratory throughput while conserving reagents by scaling the purification chemistry automatically to each sample's input volume, providing DNA that is ready for storage or for use in downstream applications without a need to resuspend DNA pellets.

Promega has developed the ReliaPrep™ Large Volume HT gDNA Isolation System to meet the demand of laboratories processing large numbers of large-volume (3–10 ml) blood samples. Large-volume sample processing for nucleic acid purification can be a tedious process that requires multiple pieces of instrumentation and many steps, particularly when precipitation-based methods or paramagnetic particles are used. The Promega ReliaPrep™ Large Volume HT gDNA Isolation System integrated on the Hamilton MICROLAB® STARplus liquid-handling workstation provides a dependable system for isolating genomic DNA from 3–10 ml of blood. The integrated system resolves challenges that researchers encounter when processing large-volume samples in a high-throughput format, such as loss of sample pellets during decanting, transport of tubes to locations on a liquid-handling robot, and resuspension of DNA pellets. Liquid-handler resource constraints were removed by the creation of a new accessory, the ReliaPrep™ HSM 32 LV Instrument, which performs heating, shaking and magnetization of samples at one deck position. The combination of this new device, the MICROLAB STARplus workstation and the ReliaPrep™ Large Volume HT gDNA Isolation System allows automated recovery of pure gDNA from up to 96 10-ml blood samples in 8 h.

### Simplified sample processing

Promega has developed a new accessory device, the ReliaPrep™ 32 LV HSM Instrument, to streamline processing of large-volume samples. This instrument eliminates the need to physically move processing tubes during DNA isolation, as it carries out three functions at one position: heating, shaking and application of a magnet to samples (**Fig. 1**). Multiple HSM instruments can be integrated on a liquid

handler, allowing processing of up to 96 samples per instrument in <8 h. Processing begins with the transfer of blood samples in 50-ml processing tubes, after which sample information (such as a barcode) is associated with each sample's position on the instrument. The elution volume for each sample is also supplied at setup. Tip use is efficient, with fewer than three tips used for each sample processed. When the ReliaPrep™ system is integrated on a liquid handler, such as the Hamilton Robotics MICROLAB STARplus, processing is automatically scaled for each sample's input volume, using liquid level detection and instrument software to conserve reagents and ensure optimal performance. The instrument and integrated method process 1–96 samples with minimal user intervention. The HSM instrument also allows for semi-automated processing of samples, during which a user dispenses the reagent and aspirates waste as directed by prompts on the HSM instrument LCD screen. In this manual mode, 32 samples can be processed in <4 h.



**Figure 1** | The ReliaPrep™ 32 LV HSM Instrument.

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## APPLICATION NOTES

**Table 1** | Average yield and purity from different sample sources and volumes

Sample	Input volume (ml)	Average yield ( $\mu\text{g}$ ) <sup>a</sup>	$A_{260}/A_{280}$	$A_{260}/A_{230}$
Whole blood	10	300	1.9	2.2
	6	180	1.9	2.0
	3	90	1.9	2.0
Buffy coat <sup>a</sup>	1.5 (15 ml blood)	458	1.9	2.2
Oragene®•DNA saliva collection device <sup>a</sup>	4.0	39 <sup>b</sup>	1.8	2.0

<sup>a</sup>gDNA recovery can vary between individuals owing to inherent variability in white blood cell count and saliva samples. <sup>b</sup>There can be large differences between individuals for saliva DNA.

### Superior performance with compromised samples

The ReliaPrep™ Large Volume HT gDNA Isolation System enables users to purify nucleic acid from the entire whole-blood sample, unlike precipitation-based methods, which begin with red blood cell lysis and purify DNA from only the white blood cell fraction. During shipping and storage, whole blood may be exposed to elevated or freezing temperatures, which can cause white blood cell lysis. When precipitation-based chemistry is used, any DNA from lysed white blood cells is lost during the red blood cell lysis step. This can result in gDNA recovery that is 50–80% lower than that from fresh whole-blood samples. Because DNA is purified from the entire whole-blood sample, the ReliaPrep™ Large Volume HT gDNA Isolation System recovers the DNA from compromised white blood cells, resulting in similar yields from both fresh and compromised whole-blood samples, with comparable purities. With the ReliaPrep™ Large Volume HT gDNA Isolation System, you can be sure of recovering gDNA from your samples whether they are fresh or compromised.

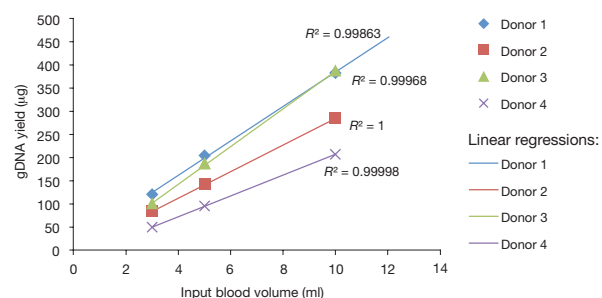
### Robust results

The ReliaPrep™ Large Volume Blood HT gDNA Isolation System isolates DNA efficiently from samples with good purity. The ReliaPrep™ resin has high DNA binding capacity and allows elution of DNA in relatively small volumes, providing DNA at a concentration suitable for storage or downstream assays. gDNA yields of ~300  $\mu\text{g}$  are obtained from 10 ml of blood with an average white blood cell (WBC) count of  $6 \times 10^6$ – $7 \times 10^6$  WBC  $\text{ml}^{-1}$ , and DNA is eluted into a volume of 500  $\mu\text{l}$  to 1.5 ml. The DNA routinely has purity ratios of  $A_{260}/A_{280} > 1.7$  or  $A_{260}/A_{230}$  of 1.8–2.2. The chemistry is scalable, offering linear recovery of DNA from input blood volumes of 3–10 ml (**Fig. 2**) with similar gDNA purity throughout the range of input volume. Eluted gDNA is suitable for storage or common downstream analyses such as PCR, genotyping, microarray analysis and other applications. Additionally, the ReliaPrep chemistry provides good recovery from fresh as well as frozen or hemolyzed blood samples, in contrast to precipitation-based methods, which have lower yields owing to premature lysis of lymphocytes. An optional RNase digestion step may be included for applications or sample types that have problems with RNA contamination.

In addition to whole blood, other sample types also have been tested. Buffy coat preparations from 10 ml of blood have been processed

using the ReliaPrep™ Large Volume HT gDNA Isolation System, providing yields similar to or better than those of precipitation-based chemistries with the same sample (**Table 1**). The system also has been used for gDNA isolation from human saliva samples collected using the Genotek Oragene®•DNA sample collection devices. Yields from 5 to 40  $\mu\text{g}$  of genomic DNA with  $A_{260}/A_{280}$  purity ratios of 1.7–1.9 have been obtained from Oragene saliva samples. Processing of saliva samples typically includes an RNase treatment owing to the greater amounts of RNA in saliva samples.

The combination of new gDNA purification chemistry, a new instrument accessory and integration onto a large liquid handler allows for walk-away high-throughput purification of gDNA from large (3–10 ml) samples. The ReliaPrep™ Large Volume HT gDNA Isolation System is an attractive option for laboratories seeking to automate gDNA isolation: it offers scalable purifications, robust performance from pristine as well as hemolysed samples, and gDNA well suited for demanding downstream applications.



**Figure 2** | gDNA was isolated from 3, 5 and 10 ml of blood from four individuals. DNA was quantified by absorbance spectroscopy, demonstrating linear scalability of the purification method.

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