

Using the Wizard® Genomic DNA Purification Kit with 96 Well Plates



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Many procedures in molecular biology require the isolation of high-quality genomic DNA; however, the starting material and sample size can vary greatly. To allow for greater flexibility, the Wizard® Genomic DNA Purification Kit protocol can be modified so that sample volumes of 50µl or less can be purified using a 96 well plate format. In this article we present information and a protocol for the purification of Genomic DNA in such a format.

INTRODUCTION

The rapid, safe isolation of high-quality genomic DNA is an important aspect of biotechnology and molecular diagnostics. The Wizard® Genomic DNA Purification Kit was designed with the flexibility to isolate double-stranded, high molecular weight genomic DNA from a variety of sources. This flexibility also allows the use of widely scalable volumes and is based on minor modifications of one central protocol. Table 1 shows DNA yields from various starting materials. The Wizard® Genomic DNA system allows purification of high-quality genomic DNA in less than 45 minutes without the use of hazardous organic solvents such as phenol or chloroform.

The largest Wizard® Genomic DNA Purification Kit, sufficient for the purification of 1 liter of blood, provides great versatility. The isolation of genomic DNA can be performed from a wide variety of tissues over a wide range of tissue amounts, as well as from any volume of blood from 20µl to 10ml. Blood preserved by using the anticoagulants EDTA, heparin or citrate, work equivalently in all Wizard® Genomic DNA blood purification protocols.

WIZARD® GENOMIC DNA PURIFICATION KIT IS FULLY SCALABLE

To facilitate the use of the Wizard® Genomic DNA system for smaller sample volumes, a 96 well purification protocol has been developed for DNA purification from 50µl or less of whole blood (Figure 1). Using this protocol, high molecular weight DNA can be recovered and visualized from as little as 20µl of starting material (Figure 2). The procedure can also be adapted for use in single-tube applications. While the protocol can be scaled down for use with less than 20µl of blood, the procedure then involves rather small amounts of solutions, such as 10µl of isopropanol or 5µl of DNA Rehydration Solution (for 10µl of blood), and solution evaporation is introduced as a variable. For these reasons, we recommend that the solution volumes described for 20µl of sample be used for all blood volumes less than 20µl. A protocol for small-scale purification in a 96 well format can be found at at:

www.promega.com/tbs/.

REHYDRATION OF GENOMIC DNA

Using the 96 well plate protocol, more than 50% of the DNA is rehydrated in the provided DNA Rehydration Solution after 20 minutes at room temperature. For applications such as DNA amplification where single-stranded DNA is used as template, additional convenience can be provided by the rehydration of genomic DNA using sodium hydroxide followed by neutralization prior to amplification. Using the sodium hydroxide/neutralization procedure, more than 80% of the purified DNA is rehydrated within 20 minutes at room temperature. Table 2 shows the relative percent of DNA in solution following DNA rehydration under various conditions. All the DNA obtained from these rehydration conditions was found to be suitable for PCR^(a) (Figure 3).

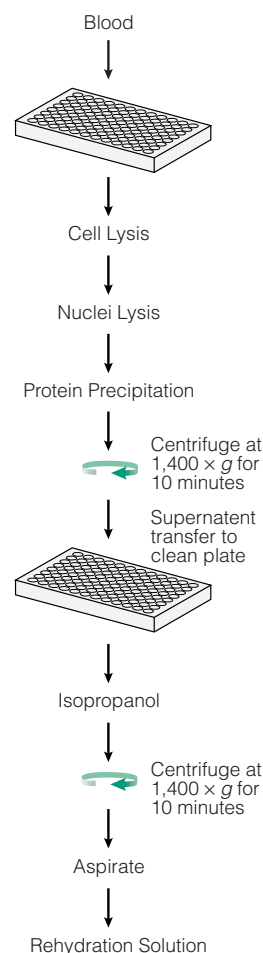


Figure 1. Schematic of the 96 well protocol for the Wizard® Genomic DNA Purification Kit.

Table 1. DNA Yields from Various Starting Materials.

Source	Amount of Starting Material	Typical DNA Yield	RNase Treatment
Human Whole Blood			
	300µl	5–15µg	Optional
	1ml	25–50µg	Optional
	10ml	250–500µg	Optional
96 well plate*	50µl/well	0.2–0.7µg	Optional
Mouse Whole Blood			
EDTA (4%) treated	300µl	6µg	Optional
Heparin (4%) treated	300µl	6–7µg	Optional
96 well plate	50µl/well	0.2–0.7µg	Optional
Cell Lines			
K562	3 x 10 ⁶ cells	15–30µg	Required
COS	1.5 x 10 ⁶ cells	10µg	Required
NIH3T3	2.25 x 10 ⁶ cells	9.5–12.5µg	Required
PC12	8.25 x 10 ⁶ cells	6µg	Required
CHO	1–2 x 10 ⁶ cells	6–7µg	Required
Animal Tissue			
Mouse Liver	11mg	15–20µg	Required
Mouse Tail	0.5–1cm of tail	10–30µg	Optional
Insects			
Sf9 Cells	5 x 10 ⁶ cells	16µg	Required
Plant Tissue			
Tomato Leaf	40mg	7–12µg	Required
Gram-Negative Bacteria**			
<i>E. coli</i> (JM109)	1ml	20µg	Required
~2 x 10 ⁹ cells/ml	5ml	75–100µg	Required
<i>Enterobacter cloacae</i> ,	1ml	20µg	Required
~6 x 10 ⁹ cells/ml	5ml	75–100µg	Required
Gram-Positive Bacteria**			
<i>Staphylococcus epidermis</i>	1ml	6–13µg	Required
~3.5 x 10 ⁸ cells/ml			
Yeast**			
<i>Saccharomyces cerevisiae</i>	1ml	4.5–6.5µg	Required
~1.9 x 10 ⁸ cells/ml			

*Yield depends upon the quantity of white blood cells present. See Table 2.
 **Overnight culture.

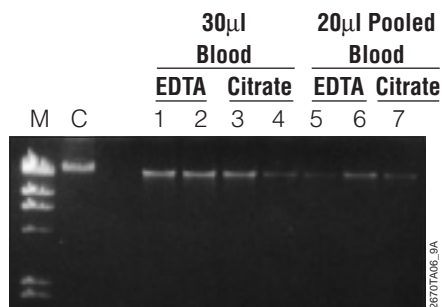


Figure 2. DNA purified with the Wizard® Genomic DNA Purification Kit using the 96 well plate protocol. DNA was prepared from different sample sizes of blood collected in either EDTA or citrate and rehydrated overnight. Lane M contains Lambda DNA/*Hind* III Markers (Cat.# G1711); lanes 1–2, 30µl in EDTA; lanes 3–4, 30µl in citrate; lanes 5–6, pooled DNA from 20µl blood in EDTA; and lane 7, pooled DNA from 20µl blood in citrate.

HIGH MOLECULAR WEIGHT DNA READY TO USE

The Wizard® Genomic DNA system produces high-quality DNA >50kb in size, which can be used for restriction enzyme analysis and applications such as Southern blotting. Figure 4 shows DNA purified from 3ml of human blood that has been cut with *Hind* III or *Kpn* I, as well as uncut controls. Figure 5 shows a Southern transfer and hybridization using Promega's *GenePrint Light*™ YNH24 (D2S44) Alkaline Phosphatase-Conjugated Probe (Cat.# DK2631).

ISOLATION OF GENOMIC DNA FROM WHOLE BLOOD USING 96 WELL PLATES

The protocol for the system can be scaled for 20–50µl of blood. Table 3 outlines the various solution volumes used in each rehydration step. Fifty-microliter preps generally yield genomic DNA in the range of 0.2–0.7µg, depending upon the number of leukocytes in the blood sample. For a complete protocol, please refer to the *Wizard® Genomic DNA Purification Kit Technical Manual* (#TM050), available online at: www.promega.com/tbs/ or by request from Promega.

Table 2. Comparison of DNA in Solution for Various Rehydration Conditions.

	Blood Volume		
	100µl	300µl	1,000µl
Rehydrate at 4°C overnight	3.1µg 100%	8.5µg 100%	35.5µg 100%
1 part 10mM NaOH for 10 minutes, then 3 parts Rehydration Buffer for 10 minutes at room temperature	87%	88%	81%
Water rehydration for 20 minutes at room temperature	42%	77%	50%
DNA Rehydration Buffer for 20 minutes at room temperature	77%	95%	53%

Values are given as percentages relative to rehydration at 4°C overnight.

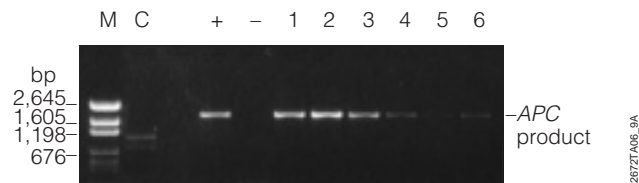


Figure 3. Comparison of rehydration methods for PCR amplification. DNA was prepared from 100µl of blood using the Wizard® Genomic DNA Purification Kit, rehydrated by different methods and then amplified by PCR. Lane M, pGEM® DNA Markers (Cat.# G1741); lane C, PCR-amplified fragment of the *APC* (*adenomatous polyposis coli*) gene as a PCR control; lane (+), positive control with 20ng human genomic DNA; lane (-), negative control. Lanes 1–2 contain DNA rehydrated overnight in DNA Rehydration Solution; lanes 3–4, DNA suspended in NaOH for 10 minutes, then DNA Rehydration Solution was added and incubated for 10 minutes; lanes 5–6, DNA suspended in water for 20 minutes.

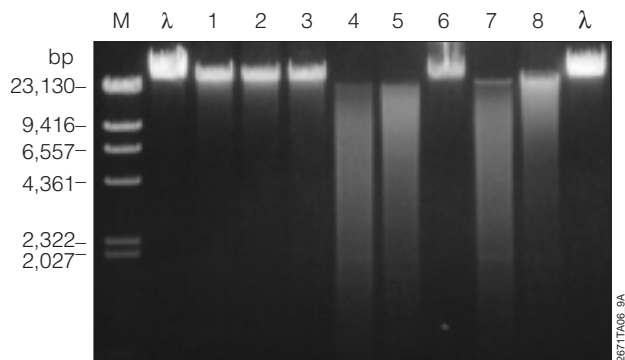


Figure 4. Suitability for restriction digest. DNA was purified using the Wizard® Genomic DNA Purification Kit and digested with either *Hind* III or *Kpn* I for 4 hours at 37°C. Lane M contains Lambda DNA/*Hind* III Markers (Cat.# G1711); lane λ, Lambda DNA (Cat.# D1501) undigested. Lane 1 contains undigested DNA stored overnight at 4°C; lane 2; undigested DNA stored overnight at -20°C; lane 3, undigested DNA in restriction enzyme buffer; lane 4, DNA digested with *Hind* III; lane 5, DNA digested with *Kpn* I; lane 6, control genomic DNA in restriction enzyme buffer; lane 7, control genomic DNA digested with *Hind* III; and lane 8, control genomic DNA digested with *Kpn* I.

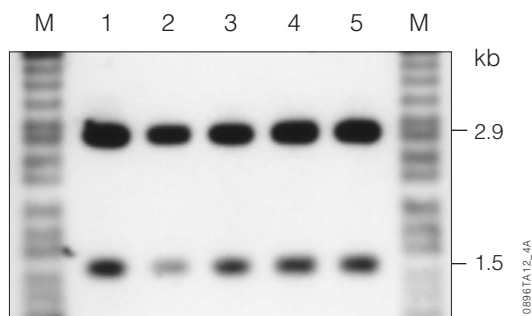


Figure 5. Southern transfer and hybridization performed with genomic DNA isolated using the Wizard® Genomic DNA Purification Kit. DNA samples were prepared as described in the text. Lanes marked M contain 10ng of the EquiLadder™ Size Marker detected with *GenePrint Light*™ EquiLadder™ System (Alkaline Phosphatase-Conjugated Probe) (Cat.# DK6030); lanes 1-5 contain 1μg of five separately isolated DNA samples digested with 1 unit of restriction endonuclease *Hae* III for 1 hour at 37°C and hybridized with the *GenePrint Light*™ YNH24 (D2S44) Alkaline Phosphatase-Conjugated Probe (Cat.# DK2631).

Table 3. Reagent Volumes Required for 20–50μl of Whole Blood.

Whole Blood	Solution				DNA Rehydration
	RBC Lysis	Nuclei Lysis	Protein Precipitation	Isopropanol	
20μl	60μl	20μl	6.7μl	20μl	10μl
30μl	90μl	30μl	10.0μl	30μl	15μl
40μl	120μl	40μl	13.3μl	40μl	20μl
50μl	150μl	50μl	16.7μl	50μl	25μl

SUMMARY

The Wizard® Genomic DNA Purification Kit is a convenient, versatile DNA purification kit that can be applied across taxa, tissue types and body fluids. Across this range of sample types, the kit can be scaled between a wide range of sample amounts (10μl–10ml of blood). Because the Wizard® Genomic DNA Purification system is scalable, there is no need for separate purchases of mini, midi and maxi kits, and the cost per preparation is directly proportional to the size of the sample and, thus, the number of samples that can be processed. The largest size Wizard® Genomic DNA Purification Kit (Cat.# A1620) can be used for all size applications, and all solutions are available separately.



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Ordering Information

Product	Size	Cat.#	Price (\$)
Wizard® Genomic DNA Purification Kit	100 × 300μl reactions	A1120	
	500 × 300μl reactions	A1125	
	100 × 10ml reactions	A1620	
Nuclei Lysis Solution	50ml	A7941	
	1,000ml	A7943	
Protein Precipitation Solution	25ml	A7951	
	350ml	A7953	
Cell Lysis Solution	1,000ml	A7933	
Rehydration Solution	50ml	A7963	
RNase A Solution, 4mg/ml	1ml	A7973	
pGEM® DNA Markers	50μg (50 lanes)	G1741	

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⁶⁹The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process.