

illustra blood genomicPrep Mini Spin Kit

Introduction

The illustra™ blood genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of high molecular weight genomic DNA (gDNA) from whole blood, buffy coat, bone marrow, and nucleated red blood cells. With the kit, gDNA can be obtained in 15 min with yields up to 23 µg, depending on source (Table 1). The procedure is rapid, but employs gentle lysis conditions that minimize shearing during the release and isolation of gDNA from blood cells.

The concentration of purified gDNA produced by the kit is suitable for use in molecular biology applications including standard, multiplex and quantitative PCR; cloning; haplotyping; restriction enzyme digestion; and genotyping.

illustra blood genomicPrep Mini Spin Kit delivers:

- **Fast results:** Convenient, streamlined workflow reduces the number of pipetting volume changes and the overall number of steps to deliver results fast—sample to gDNA in 15 min.
- **Simple flexibility:** One kit handles a wide range of blood sample types and volumes from 50 µl to 1 ml.
- **High quality:** Gentle room temperature lysis conditions deliver high-quality, > 97% intact DNA with an average size of > 20 kb.

Method overview

The illustra blood genomicPrep Mini Spin Kit uses a chaotropic agent to extract DNA from nucleated blood cells, denature protein components, and promote the selective binding of DNA to a silica membrane column (1-3). The kit completes gDNA extraction and purification in just five steps (Fig 1) and produces high quality, intact DNA. The simplified workflow is achieved by

- Conducting lysis at room temperature to gently and effectively isolate gDNA
- Eliminating the need for alcohol addition to lysate prior to column purification
- Reducing the number of centrifugation and pipetting steps
- Using consistent pipetting volumes for an efficient and easy workflow

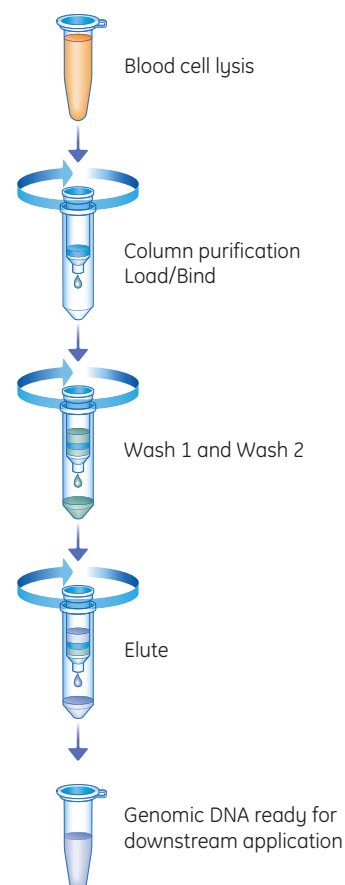


Fig 1. Schematic representation of the standard lysis method employed by the illustra blood genomicPrep Mini Spin Kit.



In addition to the standard lysis method, which is suitable for isolating gDNA from sample volumes of less than 300 μ l, the kit can also accommodate a two-step lysis method that enables the kit to extract gDNA from volumes up to 1 ml. This method is recommended for samples with cell counts greater than 1×10^8 cells/ml.

The kit also employs Proteinase K, a non-specific, subtilisin-related serine protease that is insensitive to chelating agents and detergents (4). The use of Proteinase K enables the kit to be used with a variety of fresh or frozen blood sample types, including human, horse, rabbit, rat, mouse, and chicken. The kit also includes a low ionic strength elution buffer in which gDNA can be eluted for storage.

High-quality, intact gDNA

illustra blood genomicPrep Mini Spin Kit uses a gentle lysis method that delivers intact, high-molecular-weight gDNA. Genomic DNA purified using the illustra blood genomicPrep Mini Spin Kit is typically greater than 20 kb, comprising > 97% total intact product (Fig 2). gDNA produced using the QIAamp™ Blood Mini Kit (Qiagen) only comprised 70% intact product (Fig 2).

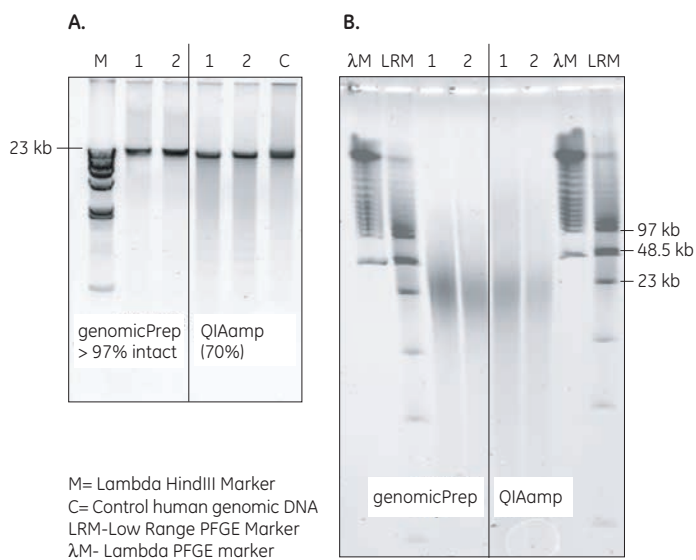


Fig 2. Genomic DNA purified from K3 EDTA human whole blood replicates using the illustra blood genomicPrep Mini Spin Kit and the QIAamp Blood Mini Kit. (A) Eluted product (3 μ l) was visualized on 1% agarose gel. C is control gDNA purified by phenol extraction. (B) Purified product (250 ng) was analyzed by pulsed field gel electrophoresis (PFGE). Genomic DNA was isolated according to manufacturers' instructions using the illustra blood genomicPrep Mini Spin Kit (GE Healthcare) and the QIAamp Blood Mini Kit (Qiagen). The illustra blood genomicPrep Mini Spin Kit is supplied with Proteinase K, which can be used with a wide variety of samples containing chelating agents and detergents. For the QIAamp Blood Mini Kit, Proteinase K was purchased separately and used per kit instructions.

Genomic DNA yield

Blood extraction with the illustra blood genomicPrep Mini Spin Kit can be completed in 15 min with yields up to 23 μ g depending on source (Table 1). Yields may vary depending on how the blood is collected and how long it has been stored. The concentration of gDNA purified using illustra blood genomicPrep Mini Spin Kit is typically 20-35 ng/ μ l, a value consistent with that needed for many downstream applications.

Table 1. Yields of gDNA from various sources obtained using illustra genomicPrep Mini Spin Kit

Sample Type (K3-EDTA anticoagulant)	Sample volume (μ l)	Yield (μ g)	Purity (A_{260}/A_{280})
Human Whole Blood	200	7.4 \pm 1.8	1.7 \pm 0.04
Horse Whole Blood	200	8.4 \pm 1.6	1.7 \pm 0.05
Rabbit Whole Blood	200	9.8 \pm 1.2	1.9 \pm 0.00
Rat Whole Blood	200	12.2 \pm 1.8	1.8 \pm 0.05
Mouse Whole Blood	200	14.1 \pm 3.5	1.9 \pm 0.05
Bone Marrow	200	23.9 \pm 2	1.9 \pm 0.06
Chicken Nucleated Blood	10	12.4 \pm 0.03	1.9 \pm 0.03

illustra blood genomicPrep Mini Spin Kit is compatible with whole blood treated with EDTA, citrate, and heparin anticoagulants (Fig 3). Yield and purity of gDNA purified using the illustra kit was superior to that obtained using the QIAamp Blood Mini Kit.

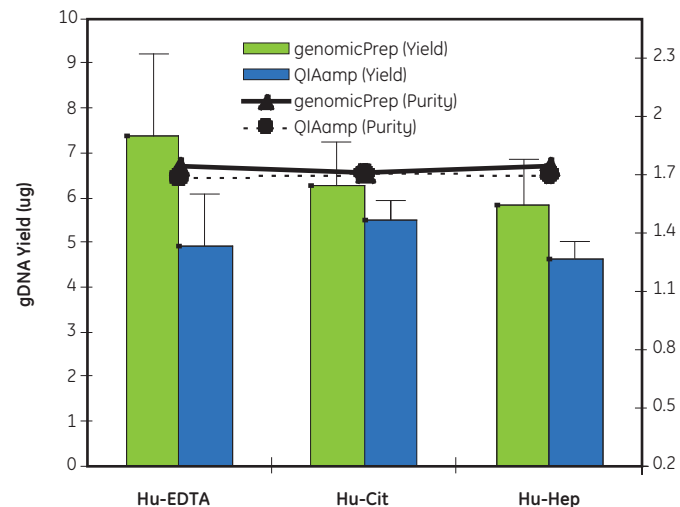


Fig 3. Yields of gDNA from 200 μ l human (Hu) whole blood treated with K3 EDTA (EDTA), sodium citrate (Cit), and sodium heparin (Hep) using the illustra blood genomicPrep Mini Spin Kit and the QIAamp Blood Mini Kit. Mean and SD are plotted. Replicates were n = 3. Product purity was > 1.7. See Fig 2 legend for gDNA isolation procedures.

Compatibility with downstream applications

Multiplex PCR

Purified gDNA produced using the illustra blood genomicPrep Mini Spin Kit is suitable for use in multiplex PCR. DNA was tested for integrity in a complex PCR design that simultaneously amplifies large and small amplicons. Three amplicons of size 335 bp, 881 bp, and 2.6 kb corresponding to CYP3A4, CYP3A5, and CYP2D6 genes were simultaneously amplified using purified gDNA from human blood (Fig 4). No under-representation or selective amplification of one particular size was observed.

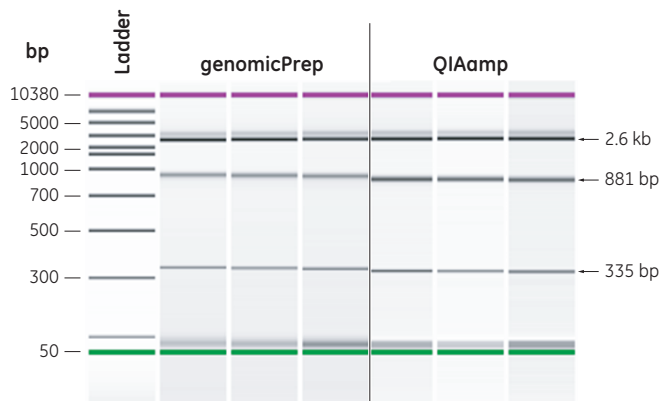


Fig 4. A representative gel image (Agilent 2100 bioanalyzer) of multiplex PCR for three amplicons corresponding to CYP3A4, CYP3A5, and CYP2D6 genes. Products purified using the illustra blood genomicPrep Mini Spin Kit and the QIAamp Blood Mini Kit were amplified in triplicate. See Fig 2 legend for gDNA isolation procedures.

Real-time PCR

illustra blood genomicPrep Mini Spin Kit works effectively with samples containing various anticoagulants and yields highly pure DNA that can be used directly in applications such as real-time PCR (Fig 5). DNA purified using the illustra blood genomicPrep Mini Spin Kit performed similarly to commercially available gDNA (correlation value of $R^2 = 0.994$). No inhibitory effects were seen in the efficiency of the PCR or the fold amplification (Ct values).

Restriction digest

The purity and concentration of gDNA isolated using illustra blood genomicPrep Mini Spin Kit enables its direct use in restriction enzyme digestions (Fig 6). Tests with over 20 restriction enzymes demonstrated that the purified DNA was free from inhibitors.

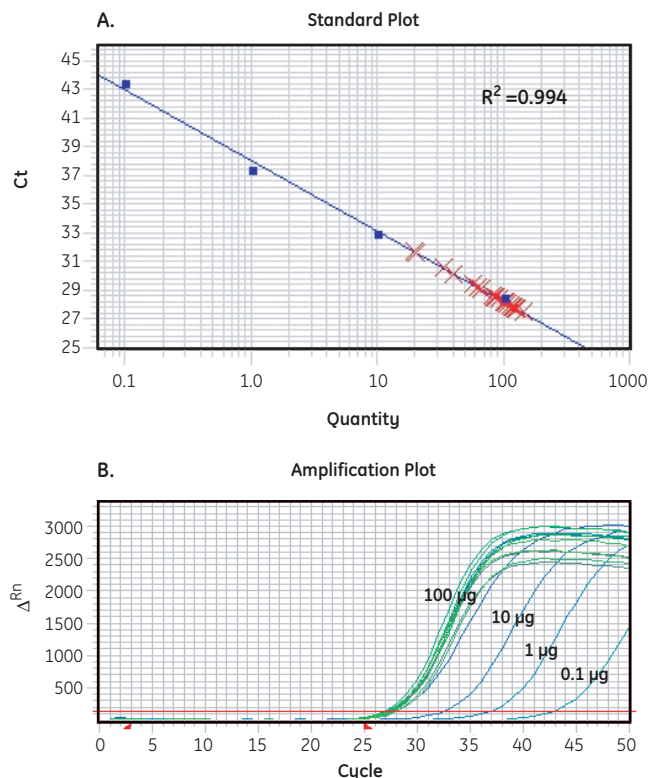


Fig 5. Real-time PCR amplification from gDNA extracted from K3 EDTA, citrate, and heparin-treated human whole blood using illustra blood genomicPrep Mini Spin Kit and QIAamp Blood Mini Kit. Replicates were $n = 3$ for each kit. The figure shows (A) a standard curve of control gDNA with all purified gDNA samples with a line fit of $R^2 = 0.994$ (in red) and (B) a representative amplification plot comparing the threshold of amplification from gDNA isolated from K3 EDTA-treated human blood using the two kits. Products purified with either the blood genomicPrep Mini Spin Kit and QIAamp Blood Mini Kit showed Ct values similar to control DNA purified by phenol extraction. See Fig 2 legend for gDNA isolation procedures. Analyses performed using an Applied Biosystems™ 7900HT Fast Real-Time PCR System.

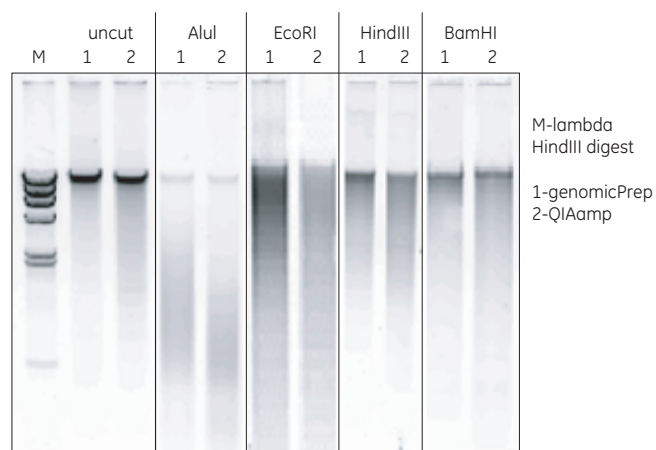


Fig 6. gDNA purified using the illustra blood genomicPrep Mini Spin Kit and the QIAamp Blood Mini Kit was digested with 22 restriction enzymes. A representative gel of AluI, EcoRI, HindIII, and BamHI digest for gDNA purified by both kit types is shown. Similar restriction enzyme activity was seen across other enzymes tested. See Fig 2 legend for gDNA isolation procedures.

Summary

The illustra blood genomicPrep Mini Spin Kit delivers high-quality intact gDNA from a variety of blood sources using a convenient and efficient protocol. The gentle lysis conditions yield gDNA that is > 20 kb and > 97% intact. The kit features a simplified protocol that minimizes pipetting volume changes and reduces the overall number of steps. The kit delivers high yields of highly pure gDNA that can be used directly in downstream applications such as multiplex, real-time, and end-point PCR, as well as restriction enzyme digestions.

References:

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Ordering information

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(50 preps)

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