Sera-Xtracta™ Genomic DNA Kit

User Guide

DOMINIQUE DUTSCHER SAS

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1 Introduction

Product code

29429140

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Storage

All kit components should be stored at room temperature (15°C – 30°C). Once reconstituted, store Proteinase K chilled (2°C – 8°C).

Expiry

For expiry date, refer to outer packaging label.

Proteinase K solution (reconstituted in DNase-free water) is stable for 4 months when stored chilled (2°C– 8°C).

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2 Components

Kit contents

Pack size: Standard pack for 96 Purifications

Product code: 29429140

Component	Amount
Proteinase K	60 mg (lyophilized)
Lysis buffer	22 mL
Magnetic silica beads	1.6 mL
Binding buffer	26 mL
Wash 1 buffer AQ	120 mL
Wash buffer 2 AQ	30 mL
gDNA Elution buffer	14 mL

Materials to be supplied by the user

Chemicals:

- DNase-free water
- Absolute ethanol

Equipment needed

- Microcentrifuge that accommodates 2.0 mL microcentrifuge tubes
- Standard laboratory shaker / mixer, for example the Eppendorf™ Thermomixer™ – setup as required to accommodate 2.0 mL microcentrifuge tubes.
- Vortex mixer
- 2.0 mL microcentrifuge tubes
- Pipette tips with aerosol barrier
- Magnetic rack, to fit 2.0 mL microcentrifuge tubes

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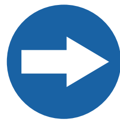

NOTICE

All tubes and pipette tips should be DNase-free grade. The working environment should also be subject to cleaning procedures to minimize the presence of extraneous DNases and (human) genomic DNA, originating from operators, samples and other biological sources.

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3 Description

Introduction

The **Sera-Xtracta Genomic DNA Kit** is designed for the rapid extraction and purification of genomic DNA from whole blood. The protocols have been designed to minimize shearing, resulting in high quality intact genomic DNA. The procedure can be completed in less than 90 min and generates DNA yields of 4 – 8 µg from 200 µL starting volume with a purity ratio (A260/A280) greater than 1.7 and minimal carryover of RNA. The kit is optimized for processing 50–200 µL of whole blood, to yield genomic DNA with a purity and quality that is compatible with most molecular biology techniques, including cloning, restriction enzyme digestion, PCR amplification, genotyping applications and next-generation sequencing. The kit contains enough reagents for 96 purifications (Product Code: **29429140**).

The developed method uses chaotropic agents to extract DNA from blood cells, denature protein components and promote the selective binding of DNA to the silica-coated magnetic beads. Proteinase K is the protease of choice to digest protein from samples, because it is active even when enzyme inhibitors such as EDTA and detergents are present in samples. Denatured contaminants are easily removed by subsequent washing of the silica beads with an ethanolic buffer set. The purified genomic DNA is eluted in a low ionic strength buffer at a concentration suitable for most downstream molecular biology applications.

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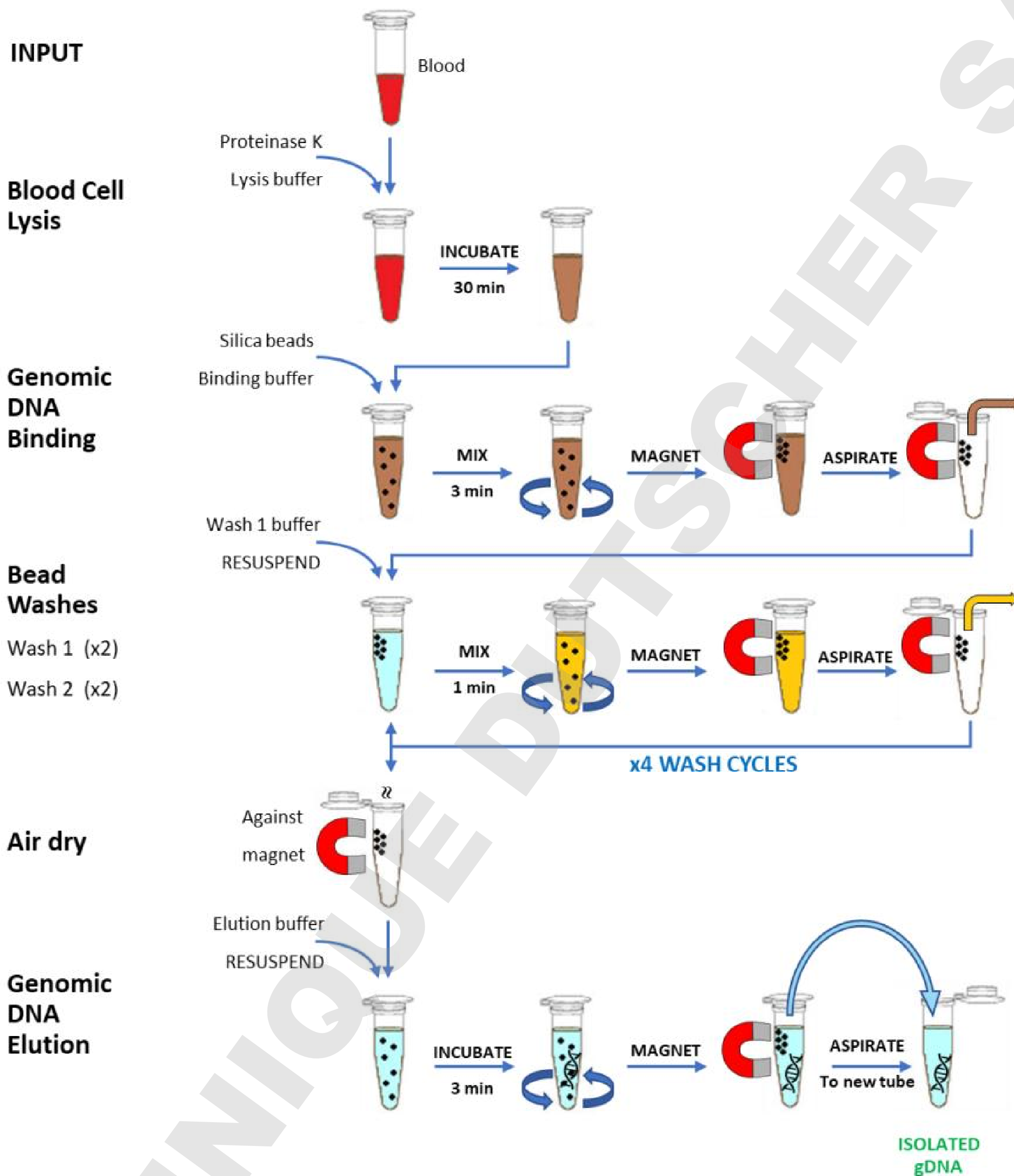
3.1 The basic principle

Illustrated procedure



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Step procedure

Use of the **Sera-Xtracta Genomic DNA Kit** involves the following steps:

Step	Comments	Component
1. Blood cell lysis	Blood cells are lysed by a chaotropic salt and detergents in the Lysis buffer, in the presence of Proteinase K.	Lysis buffer Proteinase K
2. Genomic DNA binding	The additional agents in the Binding buffer promote selective binding of genomic DNA to the magnetic silica beads. Denatured proteins are removed by aspiration of the cleared supernatant after magnet bead collection.	Silica magnetic beads Binding buffer
3a. Wash 1 buffer ×2	Wash buffer 1, containing a chaotropic salt, removes protein and other contaminants from bead-bound genomic DNA.	Wash 1 buffer
3b. Wash 2 buffer x2	Wash buffer 2 containing ethanol removes residual Wash 1 buffer salts and other contaminants.	Wash 2 buffer
4. Drying	Excess ethanol is removed by additional aspiration and air drying.	- -
5. Elution	Genomic DNA is eluted in a low ionic strength buffer and aspirated away from the magnetic silica beads into a fresh tube.	Elution buffer

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3.2 Product specifications

The **Sera-Xtracta Genomic DNA Kit** is recommended for the isolation of genomic DNA from whole blood, amounts as indicated below.

Sample type	Whole blood (fresh, or frozen / thawed)
Sample input volume	50 – 200 µL (standard input 200 µL)
Elution volume	100 µL[1]
Maximum binding capacity	~12 µg per 15 µL bead mix
Yield	4 – 8 µg of gDNA per 200 µL whole blood input
Purity (A_{260}/A_{280})	> 1.7
Product size	> 40 kbp

[1] Recommended elution volume: the end user has the option to vary this according to downstream application requirements.

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3.3 Typical output

Cell numbers

The normal healthy human blood cell count for white blood cells is $4 – 7 \times 10^6$ cells / mL. This corresponds to theoretical yields of ~ 20 - 40 µg genomic DNA / mL, which equates to a maximal achievable level of ~ 4 - 8 µg of genomic DNA for a 200 µL aliquot. The method of collecting blood and the length of storage can influence the viability of the white blood cells. The yield of genomic DNA purified using this kit is directly proportional to the quality of the input sample. Higher cell numbers can be obtained by using leukocyte-enriched fractions of whole blood or Buffy coat. Some protocol optimization may be necessary for obtaining high yields and purity of isolated genomic DNA in these cases.

UV/VIS Spectroscopy

Aqueous solutions of dsDNA are expected to show an absorbance peak at around 260 nm. As a general guideline, a solution of pure dsDNA at a concentration of 50 ng/µL is expected to show an absorbance of 1.0 AU at this spectral peak (for a typical sample pathlength of 10 mm).

As a guide to purity, two absorbance ratios are normally considered:

- A_{260}/A_{280} ratio. Commonly considered, as proteins (most likely contaminants from the original input) have a peak UV absorbance around 280 nm.
- A_{260}/A_{230} ratio. For detection of contaminating species with significant UV absorbances at this shorter wavelength (including many reagents employed for DNA isolation and purification).

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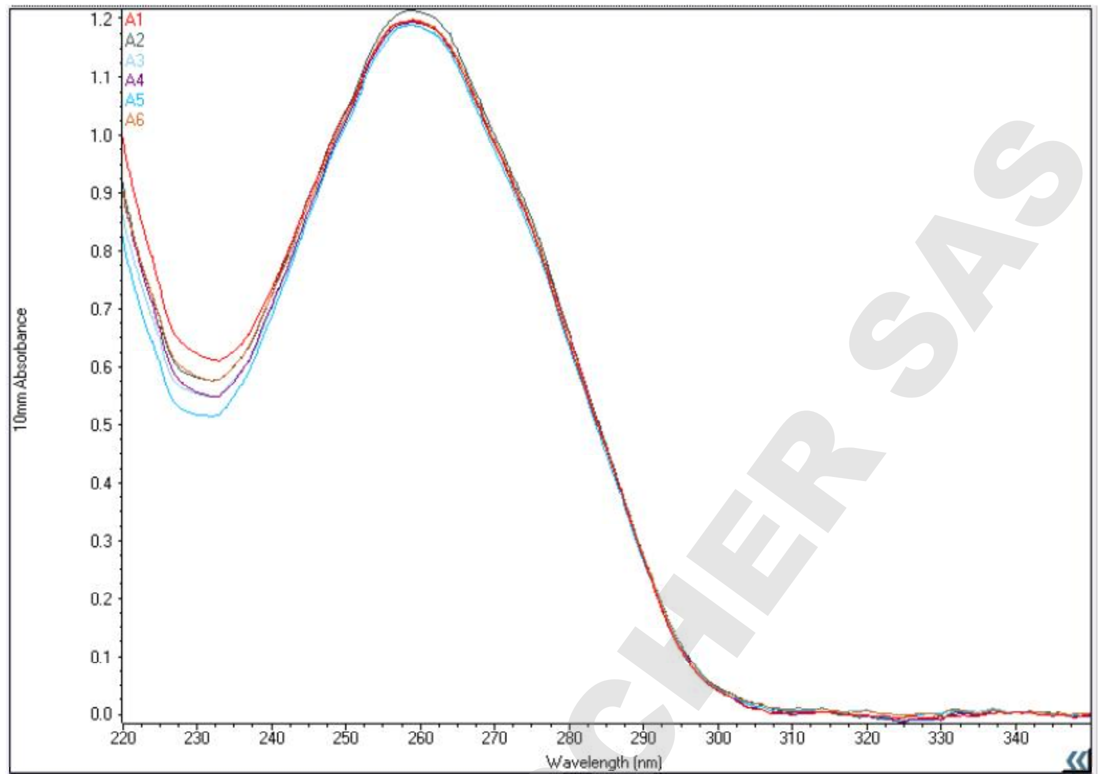

Figure 3.1: Typical UV absorbance spectra of genomic DNA isolated from EDTA-treated human whole blood.

Example absorbance spectra from 6x replicate extractions shown – 100 µL extracts from 200 µL input blood volumes. Spectra recorded via Nanodrop 2000, Nucleic Acid program, blanked on Elution buffer.

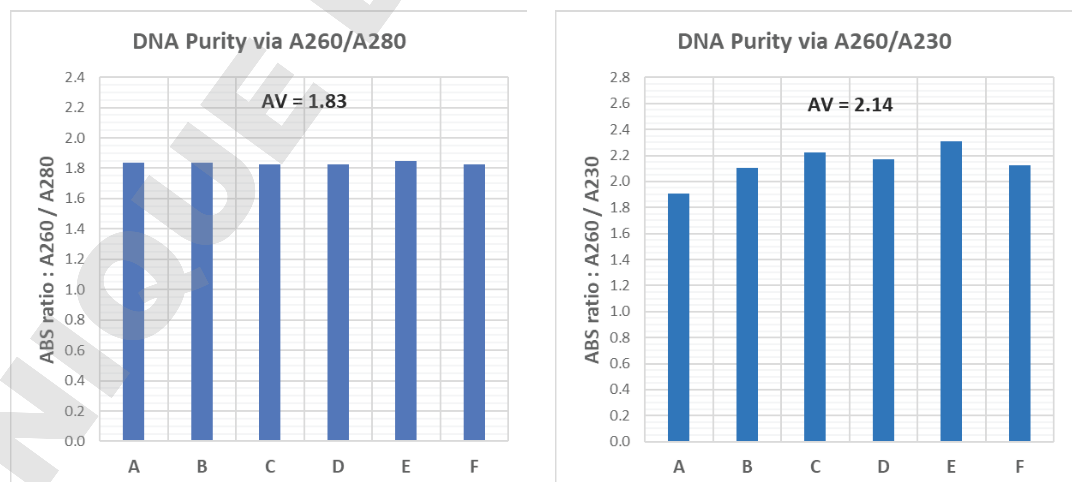

Figure 3.2: UV/VIS purity ratios of genomic DNA purified from EDTA-treated human whole blood

Absorbance purity ratios, determined as averages from 3x triplicate readings of each replicate extraction in Figure 3.1 above. The average values across all replicates is also shown.

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Fluorescence-based DNA quantitation

Quantitation of DNA via UV/Vis absorbance at 260 nm can be easily disrupted by the presence of RNA and other contaminants that also absorb around this region. As a result, techniques that offer more selectivity for double stranded DNA are preferred, such as some fluorescence-based methods e.g., PicoGreen™ dye reagent.

A commonly-used benchtop fluorescence-based quantitation system for DNA is the Qubit™ fluorimeter (Thermo Fisher Scientific), for which there are commercial detection kits for nucleic acids. For purified genomic DNA obtained using Sera-Xtracta Genomic DNA kit, the Qubit dsDNA Broad Range assay kit is recommended.

dsDNA concentration levels measured by this assay method are normally comparable to those obtained via UV/Vis spectroscopy following high quality DNA purification.

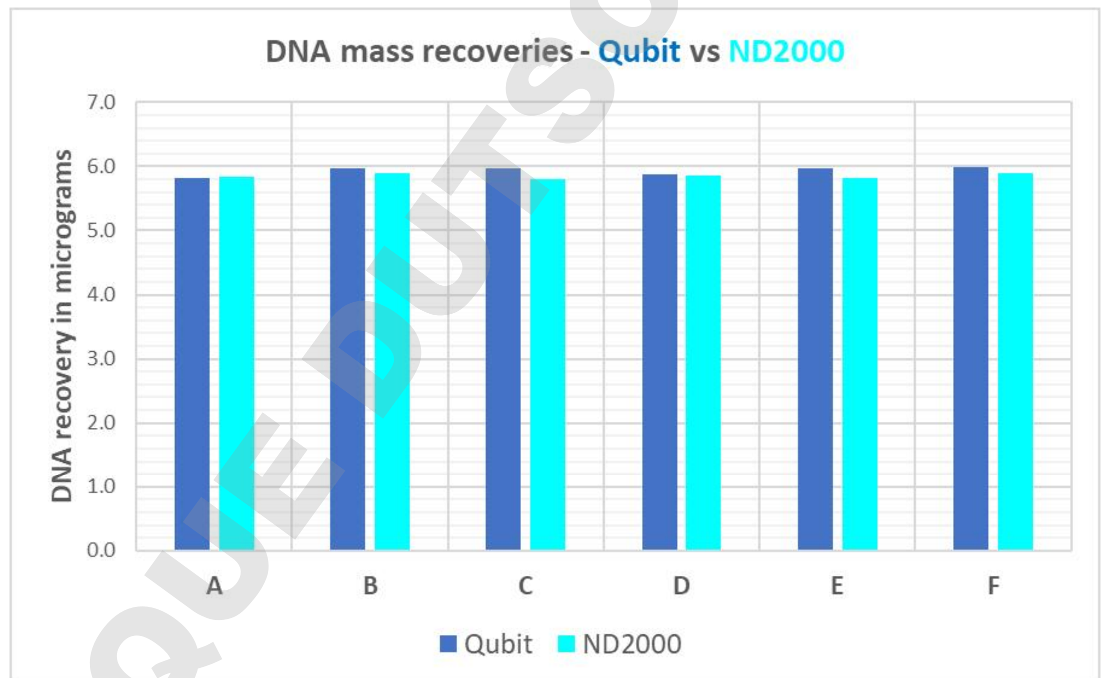

Figure 3.3: Typical mass recoveries of genomic DNA, from replicate 200 µL inputs of blood.

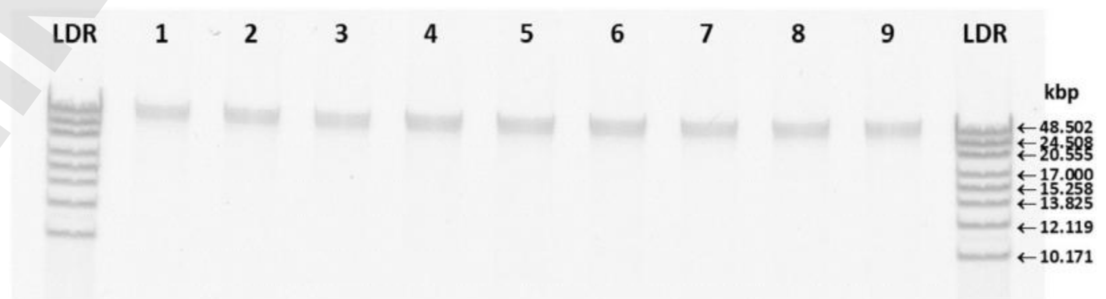

Figure 3.4: Gel characteristics of genomic DNA purified from EDTA-treated human whole blood.

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0.8% agarose gel in 1× TAE buffer. Detection via Gel Red fluorescent dye. Gel image captured on Typhoon™ imager.

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4 Protocol

Numerous factors can affect the quantity and quality of the isolated genomic DNA from blood. These factors are outlined in detail in the *Chapter 5 Appendices, on page 21*

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4.1 Preparation of working solutions and Sample collection

Preparation of working solutions

See *Chapter 2 Components, on page 4*. for Materials and Equipment to be supplied by the user.

Proteinase K

Dissolve the supplied lyophilized Proteinase K (60 mg lyophilized unit) in DNase-free water (3.0 mL). The final concentration is 20 mg/mL. Vortex mix to dissolve. Store the solution at 2°C-8°C.

Wash 1 buffer

Prior to use, add 30 mL of Absolute ethanol to Wash buffer 1. Mix by inversion. Indicate on the label that this step has been completed. Store upright and air tight at room temperature (15°C–30°C).

Wash 2 buffer

Prior to use, add 120 mL of Absolute ethanol to Wash buffer 2. Mix by inversion. Indicate on the label that this step has been completed. Store upright and air tight at room temperature (15°C–30°C).

Sample collection

An anticoagulant, such as heparin, citrate or EDTA, should be used when collecting whole blood. The blood may be stored chilled or frozen, but if processing frozen samples then allow them to thaw completely at room temperature before use. Whether the sample is fresh, has been stored chilled or has been thawed from frozen, ensure it is mixed by inversion, to obtain a homogeneous solution, before use.

Follow the protocol in *Section 4.2 Protocol for purification of genomic DNA from 50–200 μL whole blood, on page 16* for samples 50–200 μL in volume. If the starting sample volume is known and is less than 200 μL, it can be made up to 200 μL with a physiological buffer such as PBS. However, this is not critical: if the starting sample volume is unknown but is less than 200 μL, satisfactory results can be achieved using the 200 μL protocol without making the volume up to 200 μL with buffer.

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4.2 Protocol for purification of genomic DNA from 50–200 µL whole blood

For illustrations of the procedure, see *Section 3.1 The basic principle, on page 7* .

Blood cell lysis

Step	Action
1	Add 20 µL of Proteinase K into the bottom of a 2 mL microcentrifuge tube.
2	Add up to 200 µL of whole blood sample. **Note:** *Bulking up smaller blood inputs to a fixed 200 µL volume with e.g., PBS is not necessary, but is an option where the greatest level of control is required.* **Note:** *RNase-treatment is optional in this protocol. The kit is designed to minimize RNA carryover during the purification. If it is critical to obtain RNA-free product, treat the starting sample with RNase A prior to addition of the Lysis buffer. A final level of 1–2 mg/mL RNase is sufficient to degrade RNA (e.g., 4 µL RNase A @ 100mg/mL).*
3	Add 200 µL of Lysis buffer to the tube. Mix well by vortexing for 15 s.
4	Incubate the tube in a thermomixer (25°C, 0 rpm) for 30 min to aid lysis. Vortex the sample again for 5 s after incubation. At the end of this stage the color of the mixture will change from red to dark brown.
5	Briefly spin to bring sample to the bottom of the tube.

Genomic DNA binding

Step	Action
1	Add 15 µL of Magnetic bead suspension to the lysed sample. **Note:** *Make sure the magnetic beads are fully resuspended before aliquoting by vortexing vigorously, until a homogeneous suspension is achieved.*

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Step	Action
2	Add 230 µL of Binding buffer to the tube. Mix well by vortexing for 5 s.
3	Incubate the sample in the thermomixer (25°C, 1400 rpm) for 3 min to aid DNA binding to the beads.
4	Briefly spin to bring sample to the bottom of the tube.
5	Place the tube into a magnetic separation rack and allow 1 min for the beads to collect against the magnet.
6	Aspirate the cleared supernatant to waste. The supernatant will be dark at this point, so the bead pellet may be difficult to see. Make sure aspiration is done carefully to avoid disturbing the bead pellet.

Washes

Step	Action
1	Remove the tube from the magnet rack.
2	Add 700 µL of Wash 1 buffer to the tube.
3	Incubate the tube in the thermomixer (25°C, 1400 rpm) for 1 min, to disperse the beads and dissolve unwanted contaminants. Alternatively, vortex the tube for 5-10 s until the beads are fully dispersed.
4	Briefly spin to bring sample to the bottom of the tube.
5	Place the tube into a magnetic separation rack and allow 1 min for the beads to collect against the magnet.
6	Aspirate the cleared supernatant to waste. REPEAT STEPS 2 to 6 ONCE MORE with Wash 1 buffer (700 µL) THEN, REPEAT TWICE with Wash 2 buffer (700 µL each). ***Note:*** *FOUR wash steps are required in total.*

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Removal of residual ethanol (Optional)

Once the fourth wash step is complete and all residual wash buffer has been removed, this optional step may be performed to ensure complete removal of ethanol from the bead pellet.

Step	Action
1	Spin to bring any residual wash buffer droplets to the bottom of the tube.
2	Place the tube into a magnetic separation rack and allow 1 min for the beads to collect against the magnet.
3	Aspirate the cleared residual supernatant to waste using a small pipette tip.

Air drying

Step	Action
	Allow the opened tube to stand in the magnet rack for a further 5 min to complete air drying.

Elution

Step	Action
1	Remove the tube from the magnet rack.
2	Add 100 µL of Elution buffer to the tube. Mix well by vortexing, to ensure the bead pellet is dislodged from the tube wall and into the bulk buffer.
3	Incubate the tube in the thermomixer (25°C, 1400 rpm) for 3 min, to disperse the beads and elute off the bound genomic DNA.
4	Briefly spin to bring sample to the bottom of the tube.
5	Place the tube into a magnetic separation rack and allow 1 min for the beads to collect against the magnet.
6	Aspirate the cleared supernatant (containing the eluted, purified genomic DNA) into a fresh tube.

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Notes

A pulse spin in a microcentrifuge is strongly recommended before magnet settling, to ensure all the liquid sample in the tube is collected together in a single bulk volume at the bottom of the tube. Isolated droplets on the tube walls or trapped under the tube lid will affect results.

If air-drying of the DNA-bound beads to remove residual ethanol after washing is a concern, it is also possible to replace this step with 1 or 2 rinses with DNase-free water (700 µL). For this step, the water should be added whilst the tube is still positioned against the magnet and should be added so as not to unduly disturb the magnet-held bead pellet.

Note: *Care must be taken when performing this step. Some of the bound DNA may be eluted from the exposed surface of the beads, causing reduced DNA yield.*

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4.3 Storage of purified Genomic DNA

Purified genomic DNA may be stored at 2°C-8°C for a short period, if being used directly for analysis and/or downstream molecular biology applications.

In order to maintain a high-quality product for repeated use, aliquot and store purified isolates at -20°C or less.

The Elution buffer provided is recommended for eluting DNA, although DNase-free water can be used. DNA eluted in water is not recommended for long-term storage since it undergoes acid hydrolysis.

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5 Appendices

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5.1 Blood sample input range

The kit is designed to process starting sample volumes from 50–200 µL of whole blood using the standard method (See *Section 4.2 Protocol for purification of genomic DNA from 50–200 µL whole blood, on page 16*). Bulking of the input volumes to a fixed 200 µL volume with a compatible diluent e.g., PBS is NOT required, so the process is ideally suited for cases where the input volume is small (< 200 µL) and not exactly defined.

The purified genomic DNA shows a broadly linear increase in yield obtained in this range and are in line with expectations for the smaller input volumes, based on a control result with 200 µL blood.

DNA purity, as indicated by the A_{260}/A_{280} absorbance ratio, remains satisfactory.

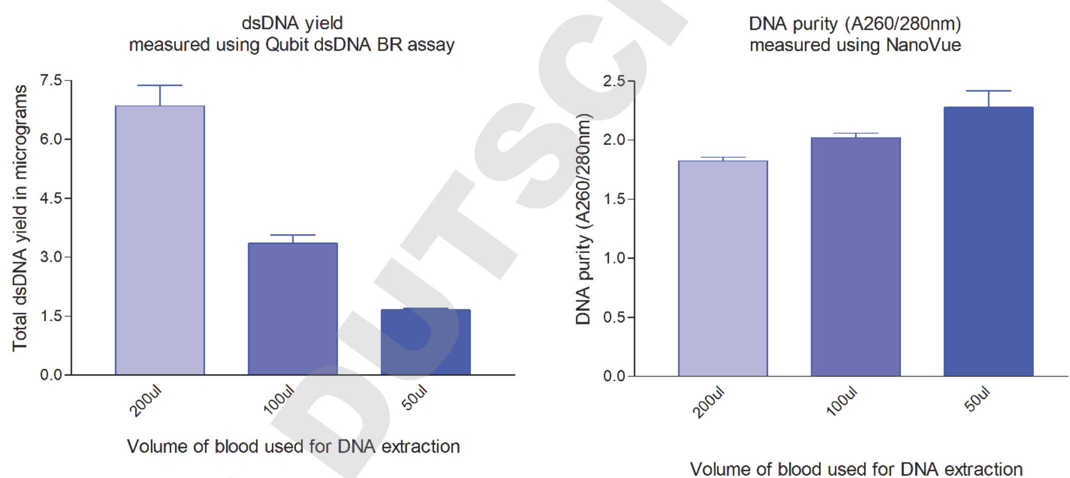

Figure 5.1: Yield of genomic DNA from 50 uL, 100 uL and 200 uL inputs of whole human blood.

Graphs show one reading taken from four replicates (n=4), error bars are +SD.

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5.2 Use of different blood collection tubes

This protocol is suitable for use with blood collected in a range of different tubes and provides the same high-quality results with no inhibitory effects downstream.

To demonstrate this, whole blood from a single donor was collected into EDTA, Heparin and Citrate blood collection tubes. DNA was isolated from 200 µL aliquots using the standard Sera-Xtracta Genomic DNA kit protocol. The purified DNA was serially diluted and subjected to real-time qPCR amplification using a kit containing a preformulated Internal PCR Control (IPC), designed to identify samples that contain PCR inhibitors (Quantifiler™ human DNA quantification kit (Thermo Fisher Scientific)).

No inhibition was observed in DNA isolated from anticoagulated whole blood collected in all three collection tubes.

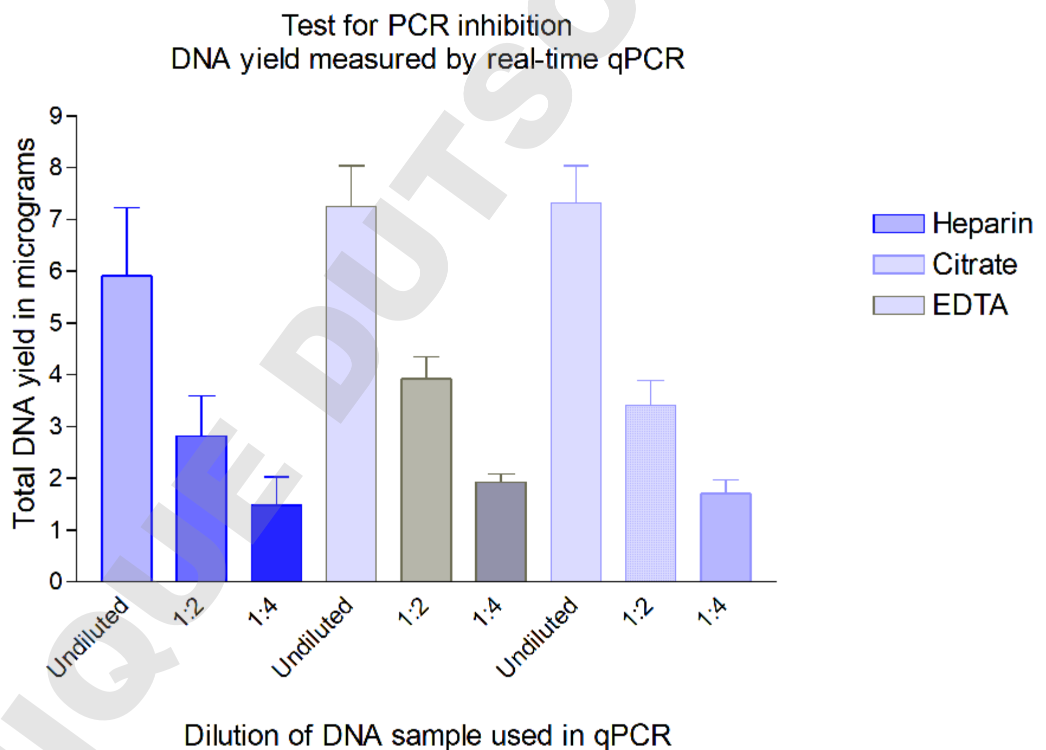

Figure 5.2: Graph shows one reading taken from six replicates (n = 6) for each sample, error bars are +SD

No inhibition was observed in samples purified from anticoagulated blood using the Sera-Xtracta Genomic DNA kit. Isolated DNA was diluted 1:2 and 1:4 to enable different volumes of eluate to be used in qPCR reactions. Results demonstrate a highly linear correlation of sample input volume with Ct values, indicating the absence of PCR inhibitors.

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There were no significant differences ($p > 0.05$, one-way ANOVA) in Ct values obtained from undiluted eluates, diluted eluates (i.e., 1:2 and 1:4 dilutions) and No Template Controls (i.e., qPCR reactions containing no DNA eluate) for the Internal PCR Control (IPC) assay (data not shown).

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5.3 Manual versus automated processing

The ability to automate the Sera-Xtracta Genomic DNA kit was demonstrated on the KingFisher™ Duo Prime System, a magnetic bead-based automation platform (Thermo Fisher Scientific).

An automation script[2] was developed for the Sera-Xtracta Genomic DNA kit based on the manual protocol (see *Section 4.2 Protocol for purification of genomic DNA from 50–200 µL whole blood, on page 16*). The resulting purifications were analysed for yield and purity.

DNA yield and purity were comparable to the manual process, as determined by the Qubit dsDNA BR assay and A260/280 nm and A260/230 nm measurement on the Nanodrop 2000.

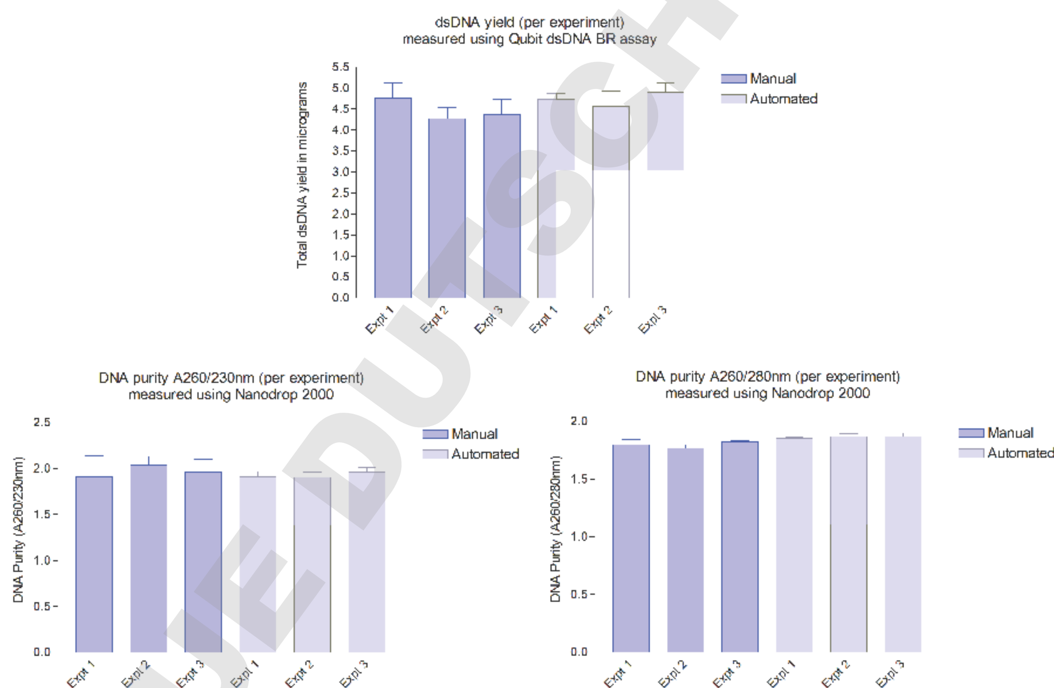

Figure 5.3: Yield and purity of double stranded genomic DNA from replicate 200 µL inputs of whole blood, comparison of manual and automated (KingFisher Duo Prime System) extraction procedures. dsDNA yield graph shows one reading taken from three replicates (n=3) for each sample. DNA purity graphs show three readings taken from three replicates (n=9) for each sample. Error bars are +SD.

Isolated DNA purity: UV absorbance ratios for the Sera-Xtracta Genomic DNA Kit are consistent and within expectations for manual and automated processes.

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2 An automation script for Kingfisher Duo is available on request from Scientific Support.(*https://www.gelifesciences.com/support/contact-us*)

5.4 Troubleshooting guide

This guide can be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare scientific support.

Visit *https://www.gelifesciences.com/en/us/support/contact-us* for contact information.

DNA yield is low

Possible cause	Suggestions
Incorrect storage of sample that resulted in degradation of DNA prior to purification.	• Blood samples should be stored with a preservative to prevent clotting and sample degradation e.g. citrate, heparin or EDTA. • Blood should be stored at 2°C-8°C for no more than 2 days before use. • Frozen blood will give slightly lower yields than fresh blood. • DNA isolations using old samples or incorrectly stored samples may not yield any DNA. • DNA yield will ultimately depend on the number of DNA-containing (nucleated) cells in the sample.
Partial loss of materials during the process when opening tubes.	• Make sure the sample volume is pooled as a single bulk volume at the bottom of the tube before opening closed tubes, otherwise sample in or around the lid may be lost.
Partial loss of magnetic beads with bound DNA during the process.	• Make sure the sample volume is pooled as a single bulk volume at the bottom of the tube before magnet settling, as beads in isolated liquid droplets cannot be properly collected and may be lost on aspiration. • Use of weak magnets or poorly-constructed racks may require longer settling times to ensure all beads are collected and so not lost during aspiration.
Wash 2 buffer was not completely removed before elution. Elevated ethanol levels in elution step inhibits DNA elution.	• Make sure that the residual traces of Wash 2 buffer and its associated ethanol content are efficiently removed as described in the drying step, before the Elution buffer is added.

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Possible cause	Suggestions
Proteinase K activity is reduced or lost.	• Proteases are essential to deproteinate the DNA prior to extraction. Reconstituted Proteinase K should be stored at 2°C -8°C and is stable for up to 4 months.

A260 / A280 of product is < 1.7

Possible cause	Suggestions
Starting sample was degraded.	• Blood and its fractions can get degraded if stored incorrectly for an extended period. Store blood at 2°C-8°C to reduce degradation of proteins and use within 2 days.
Wash and/or Wash & Dry step was incomplete.	• Repeat the Wash & Dry steps to improve purity values as described. • Take care with the wash steps to ensure all inner surfaces of the tube are exposed to wash solutions and that wash solution aspiration is as efficient as possible. This minimizes carry-over of UV-absorbing contaminants into the final DNA extracts.
Proteinase K activity reduced or lost.	• Proteases are essential to deproteinate the DNA prior to extraction. Reconstituted proteinase K should be stored at 2°C -8°C and is stable for up to 4 months.

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Final extracts contain particulates

Possible cause	Suggestions
Magnetic bead carry-over	• Make sure the sample volume is pooled as a single bulk volume at the bottom of the tube before commencing magnet settling. • Use of weak magnets or poorly-constructed racks may require longer settling times to ensure all beads are collected and so not carried over during aspiration of final extract. • Consider a more vigorous centrifuge spin before magnet settling and aspiration, to concentrate beads and other particulates into a more compact pellet. • Consider a more vigorous centrifuge spin after aspiration, before QC or onward use of extracted DNA.

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6 Related products

Product	Pack size	Product code
Sera-Xtracta Cell-Free DNA Kit	96 purifications (2 mL input)	29437807
Sera-Mag Select	5 mL 60 mL 450 mL	29343045 29343052 29343057
PuReTaq Ready-To-Go PCR Beads	Multiwell plate, 96 reactions Multiwell plate, 5 × 96 reactions 0.5 mL tubes, 100 reactions 0.2 mL hinged tube with cap, 96 reactions	27955701 27955702 27955801 27955901
GenomiPhi V2 DNA amplification kit	100 reactions 500 reactions	25660031 25660032
Ready-To-Go GenomiPhi V3 DNA amplification kit	10 purifications 100 purifications 250 purifications	28903466 28903470 28903471
GFX 96 PCR Purification Kit	96 purifications	28903445
Blood genomicPrep Mini Spin Kit	10 purifications 50 purifications 250 purifications	28904263 28904264 28904265
Tissue and cells genomicPrep Mini Spin Kit	50 purifications 250 purifications	28904275 28904276
MagRack Maxi	15 mL / 50 mL tubes	28986441
MagRack 6	1.5 mL / 2.0 mL microtubes	28948964

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7 Quick-Start Protocol

Reagent preparation before first use of kit

- Proteinase K, 60 mg – add molecular biology grade water (3.0 mL) to reconstitute the lyophilized protein to 20 mg/mL concentration. Mix to dissolve. Store reconstituted reagent at 2°C-8°C.
- Wash buffer 1 – add ethanol (30 mL) to Wash buffer 1 supplied bottle.
- Wash buffer 2 – add ethanol (120 mL) to Wash buffer 2 supplied bottle.

Required additional equipment

- Magnetic separation rack, suitable for 2mL microtubes.
- Mixer e.g., Eppendorf™ Thermomixer™ fitted with mixer head for 2 mL microtubes. Temperature set for 25°C, or use at ambient temperature (15°C-25°C).
- Bench-top centrifuge for microtubes.

Input sample

This protocol is optimized for an input sample of **200 µL blood**, to be processed in a 2 mL microtube. For different input volumes, see the full IFU online.

IMPORTANT:

After all mixing steps that contain magnetic beads (highlighted by *), the contents of the tube must be in a single bulk volume at the bottom of the tube **before** magnet settling. If this is not the case, the tube should **first** be subjected to a brief centrifuge burst in order to collect all contents into a single bulk volume.

Cell Lysis:

1. Pipette Proteinase K solution (20 µL) into the bottom of a 2 mL microtube.
2. Add whole blood aliquot (200 µL) into the microtube.
3. Add Lysis buffer (200 µL) to the sample. Vortex mix for 15 seconds, then incubate in the thermomixer (no agitation) at 25°C for 30 minutes.
   Vortex mix for 5 seconds at the end of incubation time.
4. Settle the liquids in the microtube with a brief centrifuge pulse spin before opening the tube.

DNA Binding to magnetic beads

5. Add Magnetic Bead suspension (15 µL) to the sample
   NOTE: Make sure that the magnetic beads are fully resuspended by vortexing before dispensing.
6. Add Binding buffer (230 µL) to the sample, vortex mix manually for 5 seconds, then incubate at 25°C for 3 minutes @ 1400 rpm*.
7. Place the tube into a magnet rack and allow 1 minute for the beads to collect to the magnet. Then aspirate the cleared supernatant to waste.
   NOTE: The dark colour of the lysed blood solution may render the bead pellet hard to see. Take care during aspiration to avoid disturbing the bead pellet.

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Washing of Magnetic beads

8. Remove the tube from the magnet and add the designated Wash buffer from the table below (700 µL) to the sample, then incubate in the thermomixer for 1 minute @ 1400 rpm *. Alternatively, vortex the tube for 5-10 seconds until the beads are fully dispersed.
9. Place the tube into a magnet rack and allow 1 minute for the beads to collect to the magnet. Then aspirate the cleared supernatant to waste.
10. Repeat steps 8 – 9 for the remaining rounds of washes (FOUR washes required in total).

WASH No.	1st	2nd	3rd	4th
Wash buffer ID	Wash Buffer 1	Wash Buffer 1	Wash Buffer 2	Wash Buffer 2

Drying of beads

11. Once the fourth wash step is complete and all residual wash buffer has been removed, this **optional step** may be performed to ensure complete removal of ethanol from the bead pellet.
    Optional step: Settle any residual droplets of the final wash buffer from the walls of the microtube with a brief centrifuge pulse spin. Then, place the tube back into a magnet rack and allow 1 minute for the beads to collect to the magnet. Remove any residual wash buffer supernatant from the very bottom of the tube using a small pipette tip.
12. Allow the bead pellet to air-dry for 5 minutes in the magnet rack

Elution of purified DNA from the magnetic beads

13. Remove the tube from the magnet and add Elution buffer (100 µL) to the sample, then incubate in the thermomixer at for 3 minutes at 25°C @ 1400 rpm *.
14. Place the tube into a magnet rack and allow 1 minute for the beads to collect to the magnet. Then aspirate the cleared supernatant containing the isolated genomic DNA into a fresh microtube, for QC analysis, onward useage and/or storage.

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