illustra blood genomicPrep Mini Spin Kit

Product booklet

cytiva.com

29275350 AG

Table of Contents

1 Introduction

Product codes

28904264 (50 purifications)

28904265 (250 purifications)

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.

It is the responsibility of the user to verify the use of the illustra™ blood genomicPrep Mini Spin Kit for a specific application as the performance characteristic of this kit has not been verified to any specific organism.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

WARNING
Use of this product with blood and blood products should be considered biohazardous. Follow appropriate safety procedures while using this kit and when handling DNA isolated from blood.

CAUTION

The chaotrope in Lysis buffer type 10 is harmful if ingested, inhaled, or absorbed through the skin, and can cause nervous system disturbances, severe irritation, and burning. High concentrations are extremely destructive to the eyes, skin, and mucous membranes of the upper respiratory tract.

Waste effluents from this kit should be decontaminated with bleach or a detergent-based method. Decontamination with bleach may be reactive, resulting in foam and emission of ammonia gas and should be carried out in an exhaust hood. Consult local safety regulations for safe disposal of all treated waste.

Storage

All kit components should be stored at room temperature (20°C to 25°C). Once reconstituted, store Proteinase K at 4°C.

Expiry

For expiry date please refer to outer packaging label. Proteinase K reconstituted in DNase-free water is stable for 4 months when stored at 4°C.

2 Components

Kit contents

Identification	Pack Size Product code	10 purifications Sample pack	50 purifications 28904264	250 purifications 28904265
Black	Proteinase K,lyophilized powder	1 vial (10 mg)	1 vial (30 mg)	2 vials (2 × 60 mg)
Red	Lysis buffer type 10	10 mL	50 mL	2 × 125 mL
Yellow	Wash buffer type 6	1.5 mL (Add 6 mL Absolute Ethanol before use)	6 mL (Add 24 mL Absolute Ethanol before use)	30 mL (Add 120 mL Absolute Ethanol before use)
Gray	Elution buffer type 5	3 mL	12 mL	60 mL
	illustra blood mini column	10	50	5 × 50
	Collection tubes	10	50	5 × 50

Refer to the Certificate of Analysis for a complete list of kit components.

Cytiva supplies a wide range of buffer types across the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not use the buffers supplied in other illustra kits e.g. the Lysis buffer type 10 supplied in the illustra blood genomicPrep Mini Spin Kit is not the same as the Lysis buffer type 2 supplied in the illustra blood genomicPrep Mini Spin Kit.

In order to avoid confusion and the accidental switching of buffers between kits, a numbering system has been adopted that relates to the entire range of buffers available in the illustra purification range. For example there are currently 14 Lysis buffers in the illustra range, 6 Wash buffers and 8 Elution buffers, denoted by Lysis buffer type 1 to 14, Wash buffer type 1 to 6 and Elution buffer type 1 to 8, respectively. Please ensure you use the correct type of Lysis, Wash and Elution buffer for your purification.

Materials to be supplied by user

Disposables:

1.5 mL DNase-free microcentrifuge tubes

Chemicals:

Absolute Ethanol

DNase-free water

Dulbecco's Phosphate Buffered Saline Solution (PBS) may be required.

For blood samples > 300 µL RBC lysis buffer is required as described in *Preparation of working solutions, on page 13*. For this buffer $KHCO_3$, NH_4Cl and EDTA are needed.

Equipment needed

Microcentrifuge that accommodates 1.5 mL microcentrifuge tubes

Water bath or heat-block for 70°C incubation

Vortex mixer

3 Description

Background

The illustra blood genomicPrep Mini Spin Kit is designed for the rapid extraction and purification of genomic DNA from whole blood and Buffy coat, bone marrow and nucleated red blood cells. The protocols are rapid and have been designed to minimize shearing, resulting in high quality intact genomic DNA. The kit can process 50 to 1000 µL of whole blood. Purified genomic DNA yields are typically between 4 to 6 µg from 200 µL of whole blood with a purity ratio (A_{260}/A_{280}) greater than 1.7. The procedure can be completed in less than 20 minutes to yield genomic DNA with a purity and quality that is compatible with most molecular biology techniques, including cloning, restriction enzyme digestion, PCR amplification and genotyping applications. The kit contains sufficient reagents and columns for 50 (28904264) and 250 (28904265) purifications.

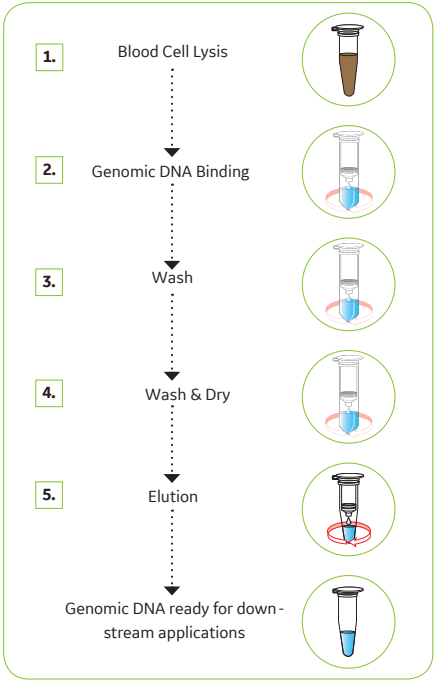
The developed method uses a chaotropic agent to extract DNA from blood cells, denature protein components and promote the selective binding of DNA to the silica-membrane contained in an illustra blood mini column (see references 1 to 3 in *Chapter 7 References, on page 36*). Proteinase K is the protease of choice to digest protein from samples, because it

is active even when enzyme inhibitors such as EDTA and detergents are present in samples (see reference 4 in *Chapter 7 References, on page 36*). Denatured contaminants are easily removed by subsequent washing of the silica membrane with an ethanolic buffer. The purified genomic DNA is eluted in a low ionic strength buffer at a concentration suitable for most downstream molecular biology applications.

The basic principle

Illustration

Use of the illustra blood genomicPrep Mini Spin Kit involves the following steps:



Step procedure

Step	Comments	Component
Blood Cell Lysis	Blood cells are lysed by a chaotropic salt in Lysis buffer type 10, in the presence of Proteinase K	**Lysis buffer type 10** **Proteinase K**
Genomic DNA Binding	The chaotropic salt in Lysis buffer type 10 promotes selective binding of genomic DNA to the silica membrane. Denatured proteins are collected in the flowthrough	**illustra blood mini column & Collection tube** **Lysis buffer type 10**
Wash	Lysis buffer type 10, containing a chaoropic salt, removes protein and other contaminants from membrane-bound genomic DNA	**Lysis buffer type 10**
Wash & Dry	Wash buffer type 6 containing Ethanol removes residual salts and other contaminants	**Wash buffer type 6**
Elution	Genomic DNA is eluted in a low ionic strength buffer	**Elution buffer type 5**

Product specifications

The illustra blood genomicPrep Mini Spin Kit is recommended for the isolation of genomic DNA from blood and its cell fractions. The kit can be used to isolate genomic DNA from various sample sources and amounts as indicated below.

Sample Types:	Whole blood, Buffy coat, bone marrow cells & nucleated blood
Sample input volume	50–300 µL whole blood, Buffy coat, bone marrow cells [1] 2–10 µL nucleated blood [2] 300–1 000 µL whole blood [3]
Elution volume	200 µL
Number of steps	5
Maximum binding capacity	60 µg
Yield	4–6 µg/200 µL whole blood
Purity (A_{260}/A_{280})	> 1.7–1.9
Time/prep	less than 20 minutes
Product size	> 20 kbp
Scalability	Up to 1 mL

[1] For 50–300 µL whole blood, Buffy coat & bone marrow cells, and 2–10 µL nucleated blood - follow protocol in *Protocol for purification of genomic DNA from 50 300 µL whole blood and its cell fractions, on page 15*.

[2] Nucleated blood refers to blood derived from avian species such as chicken.

[3] For 300–1000 µL whole blood - follow protocol in *Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*.

Typical output

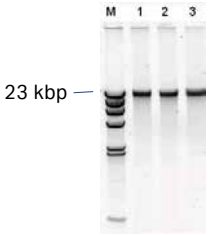


Fig 1. Gel characteristics of genomic DNA purified from K3 EDTA-treated human whole blood.

1% agarose gel loaded with 3 µL of purified eluates (n = 3).

The illustra blood genomicPrep Mini Spin Kit yields 4 to 6 µg genomic DNA from 200 µL of whole blood. The product is of high quality with purity (A_{260}/A_{280}) > 1.7 and 90% product is > 20 kbp. The purified genomic DNA is ready to use for down-stream applications like cloning, restriction enzyme digestion, PCR amplification and genotyping.

4 Protocol

Numerous factors can affect the quantity and quality of the isolated genomic DNA from blood. These factors are outlined in detail in *Chapter 5 Appendices, on page 25*.

NOTICE

Buffers and mini columns ARE NOT transferable between Cytiva illustra kits, e.g., the composition of the Lysis buffer type 10 in the blood genomicPrep Mini Spin Kit is not the same as the Lysis buffer type 2 in the bacteria genomicPrep Mini Spin Kit. Please note buffer type number for differentiation. The blood mini columns are not the same as the columns supplied in the plasmidPrep Mini Spin Kit.

Preparation of working solutions

For Materials & Equipment to be supplied by user see *Materials to be supplied by user, on page 6* and *Equipment needed, on page 7*.

Proteinase K

Dissolve the supplied lyophilized Proteinase K in DNase-free water. Add 1.5 mL of DNase-free water to the vial of Proteinase K in kit 28904264 or 3 mL to EACH vial of Proteinase K in kit 28904265. Sample pack users, please add 500 µL DNase-free water to the vial of Proteinase K. Final concentration is 20 mg/mL. Vortex to dissolve. Store the re-dissolved solution at 4°C.

Wash buffer type 6

Prior to use, add Absolute Ethanol to the bottle containing Wash buffer type 6. Add 24 mL of Absolute Ethanol to Wash buffer type 6 in kit 28904264 or add 120 mL to Wash buffer type 6 in kit 28904265. Mix by inversion. Indicate on the label that this step has been completed.

For 10 purifications sample pack size; please add 6 mL of Absolute Ethanol to Wash buffer type 6 prior to use.

Store upright and air tight at room temperature (20°C to 25°C).

Elution buffer type 5

Heat Elution buffer type 5 70°C in a water bath or a heat-block prior to start of Elution step.

RBC lysis buffer

This buffer is needed only for processing blood and/or blood fractions ranging from 300 to 1000 µL using the two-stage lysis method as described in *Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*

10 mM $KHCO_3$

155 mM NH_4Cl

0.1 mM EDTA, pH 8

Filter sterilize using a 0.2 µm filter.

Volume of RBC Lysis buffer required per purification is three times the volume of blood to be processed.

Sample collection

An anticoagulant, such as heparin, citrate or EDTA, should be used when collecting whole blood, Buffy coat and bone marrow cells. The blood may be stored at 4°C or frozen, but if processing frozen samples completely thaw at room temperature. Whether the sample is fresh, has been stored at 4°C or has been thawed from frozen, ensure complete homogenization of the sample by use of a circular wheel at room temperature for 20 to 30 minutes.

Follow the protocol in *Protocol for purification of genomic DNA from 50 300 µL whole blood and its cell fractions, on page 15* for samples 50 to 300 µL in volume but if starting sample volume is less than 200 µL, dilute to 200 µL with a physiological buffer such as PBS. When purifying genomic DNA from a sample volume of 300 to 1000 µL, follow protocol in *Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*.

Protocol for purification of genomic DNA from 50 to 300 µL whole blood and its cell fractions

Blood Cell Lysis

Step	Action
1	Add 20 µL of Proteinase K into the bottom of a 1.5 mL microcentrifuge tube.

Step	Action
2	Add up to 300 µL of whole blood sample. ***Note:*** *This protocol is suitable for 50 to 300 µL of whole blood, Buffy coat and bone marrow cells, and 2 to 10 µL of nucleated blood sample (from avian species such as chicken). Optimal performance is obtained with 200 µL whole blood. When starting volume is less than 200 µL, make input sample volume up to 200 µL with PBS.* ***Note:*** *RNase-treatment is optional in this protocol. In order to obtain RNA-free product treat the starting sample with RNase A prior to addition of the Lysis buffer type 10. A final concentration of 1 to 2 mg/mL RNase is sufficient to degrade RNA.*
3	Add 400 µL of Lysis buffer type 10 to the tube. Mix well by vortexing for 15 seconds.
4	Incubate the tube at room temperature (RT) for 10 minutes with intermittent vortexing to aid lysis. At the end of this stage the color of the reaction will change from red to dark brown.
5	Briefly spin to bring sample to the bottom of the tube.
6	Proceed with the next part of the protocol

Genomic DNA Binding

Step	Action
1	Assemble a mini column in the supplied Collection tube. Use individual columns for individual samples. These columns are for single-use only.
2	Load the complete lysate on to the center of the column using a pipet.
3	Close the cap of the column and transfer it to a micro-centrifuge. Spin the column for 1 minute at 11000 × g.
4	Remove the Collection tube containing the flow-through carefully without touching the base of the column. Discard the flowthrough.
5	Place the column back inside the Collection tube. ***Note:*** *See RPM calculation from RCF, on page 25 for RPM calculation from RCF.*
6	Proceed with the next part of the protocol

Wash

Step	Action
1	Add 500 µL of Lysis buffer type 10 to the column.
2	Centrifuge for 1 minute at 11000 × g. This step ensures complete cell lysis and denatures any residual proteins. Discard flowthrough.

Step	Action
3	Proceed with the next part of the protocol

Wash & Dry

Step	Action
1	Add 500 µL of Wash buffer type 6 to the column.
2	Centrifuge for 3 minutes at 11000 × g. Discard the Collection tube and flowthrough. ***Note:*** *Carefully discard flowthrough and the Collection tube. If any of the ethanolic wash solution comes into contact with the bottom of the column, discard the flowthrough and re-centrifuge for an additional 1 minute. The presence of Ethanol in the eluted genomic DNA may affect many downstream applications. The genomic DNA trapped on the silica matrix is of high purity and now ready for elution.*
3	Proceed with the next part of the protocol

Elution

Step	Action
1	Transfer the purification column into a fresh DNase-free microcentrifuge tube (user supplied).

Step	Action
2	Add 200 µL of 70°C pre-heated Elution buffer type 5 directly on to the center of the column. ***Note:*** *Pre-heat the Elution buffer type 5 to 70°C prior to use. The actual volume recovered will be 80% to 100% of the volume of buffer applied to the column. Change pipet tips between samples if applying Elution buffer type 5 to multiple samples, to reduce variation in volume of sample eluted.*
3	Incubate the column for 1 minute at room temperature. ***Note:*** *Do not incubate longer than 1 minute to get good quality genomic DNA.*
4	Centrifuge for 1 minute at 11000 × g to recover the genomic DNA.
5	Store purified genomic DNA at -20°C. For additional details see *Storage of purified Genomic DNA, on page 25*

Protocol for purification of genomic DNA from 300 to 1000 µL whole blood and its cell fractions

To isolate genomic DNA from sample volumes ranging from 300 to 1000 µL, we recommend the following protocol for use with this product. This method involves two-stage lysis of blood and its fractions as adapted from Vogelstein et al (see reference 5 in *Chapter 7 References, on page 36*). The first stage involves selective lysis of red blood cells (RBC) while white blood cells (WBC) are pelleted down. In the second stage, nucleated cells are lysed to release DNA that is then purified from the silica-membrane column.

The RBC lysis described below is performed in a larger tube (e.g., 15 mL) and the resulting WBC pellet is transferred to a 1.5 mL microcentrifuge tube for extraction.

Blood Cell Lysis

Step	Action
1	Prepare the RBC lysis buffer as described in *Preparation of working solutions, on page 13*. ***Note:*** *Components of RBC lysis buffer are not provided with this kit.*
2	Add three times the blood sample volume of RBC lysis buffer to a centrifuge tube (e.g., 15 mL tube). For example, add 3 mL of RBC lysis buffer for 1 mL of blood.
3	Transfer the one volume of whole blood or its cell fractions sample (300 to 1000 µL) to the RBC lysis buffer. Mix thoroughly by inverting the tube several times.

Step	Action
4	Incubate for 5 minutes at room temperature (RT).
5	Centrifuge the RBC lysis mixture at 500 × g for 2 minutes to pellet the WBCs.
6	Discard the supernatant (by decanting or aspiration) without disturbing the WBC pellet. Some residual fluid will remain on the sides of the tube. This residual fluid (approximately 50 to 100 µL) is needed to resuspend the cells before extraction.
7	Add another 100 to 150 µL of PBS to bring volumes up to 200 µL of cell suspension. Resuspend the cell pellet with vigorous vortexing and transfer the resuspended WBCs to a fresh 1.5 mL microcentrifuge tube.
8	Add 20 µL of Proteinase K to the resuspended WBCs. ***Note:*** *RNase-treatment is optional in this protocol. In order to obtain RNA-free product, treat the starting sample with RNase A prior to addition of the Lysis buffer type 10 . A final concentration of about 1 to 2 mg/mL RNase is enough to degrade RNA.*
9	Add 400 µL of Lysis buffer type 10 to the tube. Mix well by vortexing for 15 seconds
10	Incubate the tube at room temperature for 10 minutes with intermittent vortexing to aid lysis. At the end of this stage the color of the reaction will change from red to dark brown.

Step	Action
11	Briefly spin to bring sample to the bottom of the tube.
12	Proceed with the next part of the protocol

Genomic DNA Binding

Step	Action
1	Assemble a mini column in the supplied Collection tube. Use individual columns for individual samples. These columns are for single-use only.
2	Load the complete lysate on to the center of the column using a pipet.
3	Close the cap of the column and transfer it to a micro-centrifuge. Spin the column for 1 minute at 11000 × g.
4	Remove the Collection tube containing the flow-through carefully without touching the base of the column. Discard the flowthrough.
5	Place the column back inside the Collection tube. ***Note:*** *See RPM calculation from RCF, on page 25 for RPM calculation from RCF.*
6	Proceed with the next part of the protocol

Wash

Step	Action
1	Add 500 µL of Lysis buffer type 10 to the column.
2	Centrifuge for 1 minute at 11000 × g. This step ensures complete cell lysis and denatures any residual proteins. Discard flowthrough.
3	Proceed with the next part of the protocol

Wash & Dry

Step	Action
1	Add 500 µL of Wash buffer type 6 to the column.
2	Centrifuge for 3 minutes at 11000 × g. Discard the Collection tube and flowthrough. ***Note:*** *Carefully discard flowthrough and the Collection tube. If any of the ethanolic wash solution comes into contact with the bottom of the column, discard the flowthrough and re-centrifuge for an additional 1 minute. The presence of Ethanol in the eluted genomic DNA may affect many downstream applications. The genomic DNA trapped on the silica matrix is highly pure and now ready for elution.*
3	Proceed with the next part of the protocol

Elution

Step	Action
1	Transfer the purification column into a fresh DNase-free microcentrifuge tube (user supplied).
2	Add 200 µL of 70°C pre-heated Elution buffer type 5 directly on to the center of the column. ***Note:*** *Pre-heat the buffer for elution to 70°C prior to use. The actual volume recovered will be 80% to 100% of the volume of buffer applied to the column. Change pipet tips between samples if applying Elution buffer type 5 to multiple samples, to reduce variation in volume of sample eluted.*
3	Incubate the column for 1 minute at room temperature. ***Note:*** *Do not incubate longer than 1 minute to get good quality genomic DNA.*
4	Centrifuge for 1 minute at 11000 × g to recover the genomic DNA.
5	Store purified genomic DNA at -20°C. For additional details see *Storage of purified Genomic DNA, on page 25*

Storage of purified Genomic DNA

Purified genomic DNA may be stored at 4°C for a short period. In order to maintain a high quality product for repeated use, aliquot and store purified samples at -20°C. The Elution buffer type 5 provided should be the preferred buffer for eluting samples, although DNasefree water can be used. DNA eluted in water is not recommended for long-term storage since it undergoes acid hydrolysis (see reference 6 in *Chapter 7 References, on page 36*).

5 Appendices

RPM calculation from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

RPM = 1000 × √(RCF/1.12r)

Where RCF = relative centrifugal force; r = radius in mm measured from the center of the spindle to the bottom of the rotor bucket; and RPM = revolutions per minute.

E.g. if an RCF of 735 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3000.

Blood source, anticoagulant and cell number

Blood source

This kit performs well with whole blood and Buffy coat preparation from humans, horses, rabbits, rats, mice and chickens. Unique differences between species exist in the aggregation tendency of blood (see reference 7 in *Chapter 7 References, on page 36*) and this can affect the efficient lysis and extraction of WBC. Also fresh or frozen blood can be used to isolate genomic DNA. Both yield and purity of the end-product will be determined by the total viable WBC count in the blood samples.

See *Table 1, on page 26* for typical yield and purity of genomic DNA isolated from different species.

Table 1. Comparison of yield (µg) and purity (A260/A280) of isolated genomic DNA from whole blood from different animals

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

Whole blood and its cell fraction was collected using K3-EDTA anticoagulant across all animals. Indicated amount of blood was processed using the illustra blood genomicPrep Mini Spin Kit protocol with n = 3. Mean and SD are shown.

Blood sample input range

The kit is designed to process starting sample volumes from 50 to 300 µL of whole blood using the standard direct lysis method in *Protocol for purification of genomic DNA from 50 300 µL whole blood and its cell fractions, on page 15*. The purified genomic DNA shows a linear increase in yield obtained in this range. For nucleated blood samples a smaller sample input volume of 2 to 10 µL is recommended due to the high concentration of nucleated cells. For sample volumes between 300 to 1000 µL a modified two-stage lysis has also been developed (*Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*).

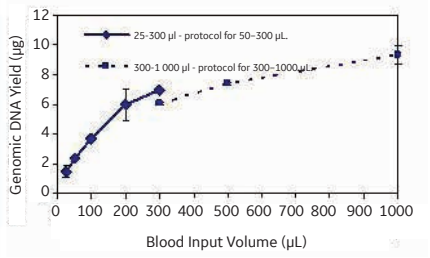


Fig 2. Human whole blood input volume versus yield using the two methods provided

K3-EDTA whole human blood sample from 25 to 1000 µL was extracted using the illustra blood genomicPrep Mini Spin Kit protocol. Replicates were n = 3. Product purity as measured by A260/A280 ratio was > 1.7. Mean and SD are plotted. Methods used were as described in *Protocol for purification of genomic DNA from 50 300 µL whole blood and its cell fractions, on page 15* and the two-stage lysis method in *Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*.

Anticoagulants

Different anticoagulant-treated whole blood such as heparin, EDTA and citrate have been shown to consistently produce high yield and purity genomic DNA with this kit. The purified product performed well when used in quantitative PCR. No differences were seen in either the change in efficiency of the PCR or the fold amplification (Ct values) compared to genomic DNA isolated by other solution-based methods.

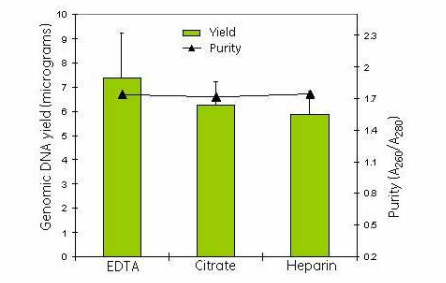


Fig 3. Similar yields of purified genomic DNA isolated from human (Hu) whole blood treated with various anticoagulants

Whole blood was collected from an individual and treated with K3-EDTA, sodium citrate and heparin, and extracted using the illustra blood genomicPrep Mini Spin Kit. Replicates were n = 3. Product purity as measured by A_{260}/A_{280} was > 1.7. Mean and SD are plotted.

Cell numbers

The normal human blood cell count for WBCs is 4500 to 11000 × 10^3 cells/mL. This corresponds to theoretical yields of 27 to 66 µg genomic DNA/ml blood (see reference 8 in *Chapter 7 References, on page 36*). For 200 µL of blood the maximal achievable yield is 5.4 to 13.4 µg. The method of collecting blood and the length of storage can influence the viability of the WBCs. 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 complete lysis of Buffy coat and for obtaining high purity product.

Lysis requirements

Cell number and scalability

This kit is designed to purify genomic DNA from 50 to 300 µL of whole blood and its fractions. The cell count of the starting material will determine the yield and purity of genomic DNA isolated from this kit. We offer a modified two-stage lysis method for samples 300 to 1000 µL as described in *Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*.

Lysis temperature and time

Under the conditions developed with this kit, 200 µL of whole human blood is completely lysed at room temperature within 10 minutes. The efficiency of lysis will change based on the cell count of the sample. The product yields and purity of genomic DNA will depend on the efficiency of the lysis reaction.

RNAase-treatment

If RNA-free samples are required, carry out the RNase-treatment during the Blood Cell Lysis step as suggested in *Protocol for purification of genomic DNA from 50 300 µL whole blood and its cell fractions, on page 15* and *Protocol for purification of genomic DNA from 300 1000 µL whole blood and its cell fractions, on page 20*.

Elution requirements

Elution temperature and time

Use Elution buffer type 5 supplied with this kit for maximal recovery. Alternatively, 10 mM Tris-HCl pH 8.0 or autoclaved double-distilled water may be used. Prior to carrying out the Elution step, pre-heat the Elution buffer type 5 or water to 70°C. Heated Elution buffer type 5 will give maximal DNA recovery. Unheated Elution buffer gives reduced product recovery of about 50°C.

Elution buffer type 5 is incubated with the silica-membrane for 1 minute at room temperature prior to collecting the purified product. Increasing this incubation time has marginal improvements in yield, but may deteriorate the purity of the collected product.

Successive elution

A single elution step recovers about 70% to 80% of the purified genomic DNA from the column. Multiple elution steps can increase the yield, but the purity of the product may be lower.

Elution volume recovery

For optimal DNA recovery, use 200 µL of Elution buffer type 5. Elution volumes of 100 µL or lower may be used to concentrate the sample, but this will reduce the yield. For example, using 50 µL instead of 100 µL will concentrate the DNA approximately 2 × with a loss in recovery of approximately 20%. The actual volume recovered will be about 80% to 100% of the volume of elution buffer applied to the column.

Product size

The rapid and gentle protocol provided with this kit isolates genomic DNA from blood and its fractions, with a characteristic band size of greater than 20 kbp. This major band constitutes greater than 90% of the purified product visible on an agarose gel.

Genomic DNA quantitation

The yield of genomic DNA isolated by use of this kit can be measured by UV absorbance. A typical concentration range is 20 to 35 ng/µL and is ample for many downstream applications. If higher concentrations are required, reduce volume of Elution buffer type 5 used to 50 to 100 µL.

Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact Cytiva technical services. Visit *cytiva.com* for contact information.

Table 2. Problem: 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 4°C for no more than 2 days. 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.
Wash buffer type 6 was not completely removed before Elution.	• Make sure that the illustra blood mini column is centrifuged for at least 3 minutes as described in Wash & Dry step before the Elution buffer type 5 is added. If humidity is high, increase the spin time to 5 minutes.
Proteinase K activity reduced or lost.	• Proteases are essential to deproteinate the extracted DNA. Reconstituted proteinase K should be stored at 4°C and is stable for up to 4 months.

Table 3. Problem: A_{260}/A_{280} 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 of time. Store blood at 4°C to reduce degradation of proteins and use within 2 days.
Wash and/or Wash & Dry step was incomplete.	• Repeat the Wash and/or Wash & Dry step to improve purity values as described.

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

Table 4. Problem: Purified genomic DNA floats out of the well when loading a gel

Possible cause	Suggestions
Wash buffer type 6 was not completely removed before Elution.	• Make sure during the Wash & Dry step the column is centrifuged for at least 3 minutes to dryness. If humidity level is high, increase the spin time to 5 minutes.
Wash buffer type 6 remained in the column below the frit and was collected in the final elution.	• The Collection tube was not emptied after the initial sample was spun through the column. This caused the Collection tube to overfill when the wash steps were performed which then caused fluid to remain in the bottom of the column. Empty the Collection tube as described in the procedure. If necessary, place the column back into the Collection tube and spin briefly to remove any residual fluid.

Table 5. Problem: Purified genomic DNA does not cut to completion with restriction enzymes.

Possible cause	Suggestions
EDTA in the Elution buffer type 5 can inhibit restriction enzymes.	• Elute the sample with heated water to facilitate complete digestion of the product or consider using another restriction enzyme.
Wash buffer type 6 was not completely removed before Elution.	• Make sure during the Wash & Dry step the illustra blood mini column is centrifuged for at least 3 minutes to dryness as described in *Protocol for purification of genomic DNA from 50 300 µL whole blood and its cell fractions, on page 15*. If humidity level is high, increase the spin time to 5 minutes.

Possible cause	Suggestions
Wash buffer type 6 remained in the column below the frit and was collected in the final elution.	• The Collection tube was not emptied after the initial sample was spun through the column. This caused the Collection tube to overfill when the wash steps were performed which then caused fluid to remain in the bottom of the column. Empty the Collection tube as described in the procedure. If necessary, place the column back into the Collection tube and spin briefly to remove any residual fluid.

6 Related products

A full range of molecular biology reagents can be found on the Cytiva website and in the catalog.

Application	Product	Product code	Pack sizes
Buffer preparation	Water, nuclease-free	US70783	500 mL
Kits containing ready-to-use mix for PCR amplification	illustra Hot Start Master Mix	25150001	100 reactions
	illustra PuReTaq Ready-To-Go™ PCR Beads	27955701	96 reactions in 0.2 mL tubes/plate
	illustra PuReTaq Ready-To-Go PCR Beads	27955702	5 × 96 reactions in 0.2 mL tubes/plate
	FideliTaq™ PCR Master Mix Plus (2 ×)	E71182	100 reactions
	FideliTaq PCR Master Mix Plus	E71183	100 reactions
Premixed nucleotides for PCR amplification	illustra DNA Polymerization Mix dNTP Set (A,C,G,T) 20 mM each	28406557	10 µmol

Application	Product	Product code	Pack sizes
	illustra DNA Polymerization Mix dNTP Set (A,C,G,T) 20 mM each	28406558	40 µmol (4 × 10 µmol)
Premixed nucleotides for PCR amplification	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 25 mM each	28406560	500 µL
	illustra PCR Nucleotide Mix dNTP Set (A,C,G,T) 2 mM each	28406562	1 mL
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 reactions
	ExoSAP-IT	US78201	500 reactions
Sequencing reaction kits optimized for MegaBACE DNA analysis system	DYEnamic ET Terminator Cycle Sequencing Kits	US81050	100 templates
	DYEnamic ET Terminator Cycle Sequencing Kits	US81060	100 templates

7 References

1. Sambrook, J., Fritsch, E.F & Maniatis, T., *Molecular cloning: A laboratory Manual*, Cold Spring Harbor laboratory, 2nd eds., (1989).
2. Marko, M.A., Chipperfield, R. & Birnboim, H.C., *Anal. Biochem*. 121,382 (1982).
3. Melzak, K. A. et al., J. Coll. Interf. Sci. 181, 635-644 (1996).
4. Hilz, H. et al. Eur. J. Biochem. 56, 103-108 (1975).

5. Vogelstein, B. & Gillespie, D., *Proc. Natl. Acad.* Sci. USA 76, 615 (1979).
6. Ausubel, F.M. et al., eds., *Current Protocols in Molecular Biology* 1, 1.68 (1991).
7. Baskurt et al., Am. J. Physiol. Heart Circ. Physiol. 273, 2604-1612, 1997.
8. *http://www.cc.nih.gov/ccc/patient_education/pepubs/cbc97.pdf*

8 Quick reference protocols

Cue card A

Quick Reference Protocol Card

illustra™ blood genomicPrep Mini Spin Kit

28904264 (50 purifications)
28904265 (250 purifications)

A. Protocol for the purification of genomic DNA from 50 to 300 µL whole blood and its cell fractions

• Elution buffer type 5, pre-heated to 70°C

:Add :Spin :Incubate

1. Blood cell lysis

- 20 µL Proteinase K
- If sample volume is less than 200 µL, make up to 200 µL with PBS
- 200-300 µL whole blood or its cell fractions
- 400 µL Lysis buffer type 10; vortex to mix
- 10 minutes room temperature
- Pulse

2. Genomic DNA binding

- Load onto assembled column and Collection tube
- 1 minute 11000 × g; discard flow through

3. Wash

- 500 µL Lysis buffer type 10
- 1 minute 11000 × g; discard flow through

4. Wash & Dry

- 500 µL Wash buffer type 6
- 3 minutes 11000 × g; discard flow through

5. Elution

- Insert column in to a clean DNase-free microcentrifuge tube
- 200 µL Elution buffer type 5, pre-heated to 70°C
- 1 minute room temperature
- 1 minute 11000 × g
- Collect eluate
- Store purified genomic DNA at -20°C

Cue card B

Quick Reference Protocol Card

illustra™ blood genomicPrep Mini Spin Kit

28904264 (50 purifications)
28904265 (250 purifications)

B. Protocol for the purification of 300 to 1000 µL whole blood and its cell fractions

- Prepare RBC lysis buffer
- Ensure Elution buffer type 5 pre-heated to 70°C

:Add :Spin :Incubate

1. Blood cell lysis

- 3 × sample volume of RBC Lysis buffer to centrifuge tube
- 300 to 1000 µL sample; mix by inverting the tube
- 5 minutes room temperature
- 2 minutes 500 × g; discard supernatant
- 100 to 150 µL PBS to bring volume to 200 µL; vortex to re-suspend pellet
- Transfer to a fresh 1.5 mL microcentrifuge tube
- 20 µL Proteinase K
- 400 µL Lysis buffer type 10; vortex to mix
- Incubate 10 minutes room temperature with intermittent vortexing
- Pulse spin.

2. Genomic DNA binding

- Load onto assembled column and Collection tube
- 1 minute 11000 × g; discard flow through

3. Wash

- 500 µL Lysis buffer type 10
- 1 minute 11000 × g; discard flow through

4. Wash & dry

- 500 µL Wash buffer type 6
- 3 minutes 11000 × g; discard flow through

5. Elution

- Insert column into a clean DNase-free microcentrifuge tube
- 200 µL Elution buffer type 5, pre-heated to 70°C
- 1 minute room temperature
- 1 minute 11000 × g
- Collect eluate
- Store purified genomic DNA at -20°C

cytiva

cytiva.com

For local office contact information, visit cytiva.com/contact

29275350 AG V:4 09/2020