SphaeraMag® Genomic DNA Blood Purification Kit

Nucleic Acid Extraction
for Manual and Automated Systems

Laboratory Use Only

Instruction Manual
Version 2.5

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Catalogue Number

Batch Code

Consult instructions for use

Manufacturer

Use by date

Temperature limit

Contains sufficient for <n> tests

1. INTRODUCTION

The SphaeraMag® Genomic DNA Blood Purification Kit is designed for rapid and reliable isolation of genomic DNA (gDNA) from blood samples (collected in anticoagulants EDTA, Heparin, Citrate). Samples are lysed in specifically formulated buffers to release nucleic acids. The gDNA is bound to the surface of paramagnetic beads, while proteins and cellular debris are removed during washing steps. The gDNA is then eluted in the provided Elution Buffer. Alternatively, nuclease-free water or other low ionic strength buffers can be used. The procedure can be performed using automated systems (like Phoenix-Pure) or manually. The purified gDNA is suitable for numerous downstream applications such as qPCR, end-point PCR, SNP analysis, genotyping, microarray analysis and library preparation for NGS sequencing.

2. KIT COMPONENTS

	SphaeraMag® Genomic DNA Blood Purification Kit - Universal 96	SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled 96	SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled 32	SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled Mini
Catalogue No.	**PCCSKU16067**	**PCCSKU16068**	**PCCSKU16069**	**PCCSKU16070**
Preps	96	96	80	48
Enhancer Solution	4 ml	Pre-filled	Pre-filled	Pre-filled
Lysis/Binding Buffer	20 ml	20 ml	20 ml	10 ml
Wash Buffer I	47 ml	Pre-filled	Pre-filled	Pre-filled
Wash Buffer II	27 ml	Pre-filled	Pre-filled	Pre-filled
Elution Buffer	10 ml	Pre-filled	Pre-filled	Pre-filled
Proteinase K	2 x 1 ml	2 x 1 ml	2 x 1 ml	1 ml
Magnetic Beads	4 x 1 ml	4 x 1 ml	4 x 1 ml	2 x 1 ml
Tip comb(s)	-	1 x 96 tip comb (in holder)	10 x 8 tip combs	30 x 8 tip combs

Table 1. SphaeraMag® Genomic DNA Blood Purification Kit content.

3. KIT COMPATIBILITY

	Universal 96	Pre-filled 96	Pre-filled 32	Pre-filled Mini
Phoenix-Pure 96/Auto-Pure 96	+	+		
Phoenix-Pure 32/Auto-Pure 32	+		+	
Auto-Pure Mini	+			+
KingFisher™ Flex Purification System	+	+		

Table 2. SphaeraMag® Genomic DNA Blood Purification Kit compatibility.

4. STORAGE AND STABILITY

Proteinase K enzyme must be stored at -25 to -15°C, Magnetic Beads at +2 to +8°C upon arrival. All other kit components are stored at room temperature (+15 to +25°C). The expiry dates of the kit and single components are stated on the outer packaging as well as on the individual reagents.

5. PRECAUTION

Proper laboratory safety practices should be employed, including the use of lab coat, gloves and protective goggles. For information regarding the composition of buffers, please check the Safety Data Sheets (available upon request). Each reagent is optimized for the specific use with this kit. Do not substitute reagents from any other manufacturer into the kit and do not combine with different Lot numbers.

6. PROCEDURE OVERVIEW

Blood samples are first treated with Proteinase K in combination with Enhancer Solution. In the following step, a specifically formulated chaotropic salt-based Lysis/Binding Buffer is used to simultaneously lyse cells, degrade protein components (including hemoglobin) and bind the gDNA to the surface of paramagnetic beads. Additionally, it offers protection against high nuclease activity.

A three step washing procedure, including 2 washing buffers, is performed to remove proteins, detergents and other impurities. The gDNA is then released from the magnetic beads using Elution Buffer.

7. ILLUSTRATED PROTOCOL

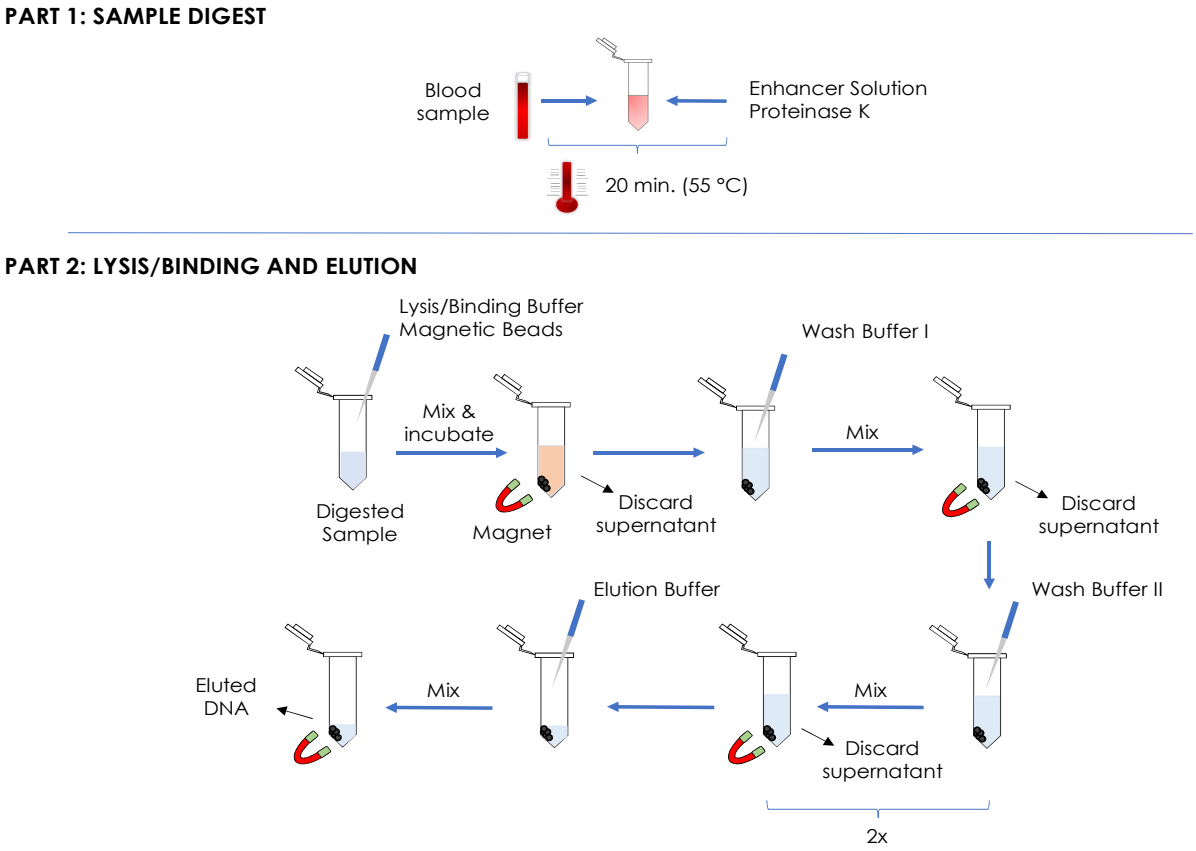


Figure 1. Illustrated protocol for manual gDNA isolation.

8. REQUIRED MATERIALS NOT SUPPLIED

General

- Ethanol, absolute (only for Universal 96)
- Isopropanol ≥99.5% (only for Universal 96)
- Suitable reagent and sample dispensing options
- Consumables for isolation devices for automated extraction
- Vortexer
- 1.5 ml or 2 ml microcentrifuge tubes (ideally low binding)

Specific for manual purification

- Thermal mixer for 1.5 ml or 2 ml tubes (or alternative heating device)
- Water bath (or alternative heating device to pre-heat buffers)
- Magnetic separation rack for 1.5 ml or 2 ml tubes

9. SPECIFICATIONS

Sample input	200 µl whole blood
Yield	6-10 µg (individual variation and anticoagulant used)
Genomic DNA Size	Peak size >50 kb
Purity	A260/280 >1.8 A260/230 >2.0

Table 3. Kit specifications.

10. PERFORMANCE AND DOWNSTREAM APPLICATIONS

The SphaeraMag® Genomic DNA Blood Purification Kit generates excellent input material regarding DNA integrity and purity for downstream applications such as quantitative PCR and NGS library preparation.

DNA Integrity

The gDNA samples were analyzed regarding DNA integrity by loading 10 µl eluate on an 1% agarose gel. Three representative samples of human whole blood collected in most commonly used anticoagulants (EDTA, Heparin, Citrate) are shown.

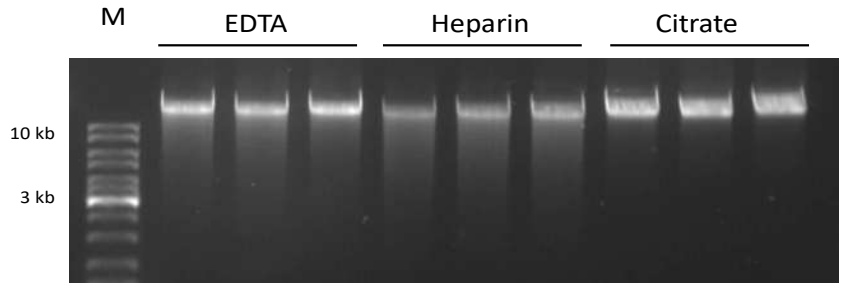


Figure 2. Integrity of gDNA isolated from whole blood samples (collected in EDTA, Heparin and Citrate collection tubes) assessed by agarose gel electrophoresis.

Quantitative PCR

Purified gDNA from representative human blood samples collected in the most widely used anticoagulants (EDTA, Heparin, Citrate) was diluted to produce a four-log range of input template concentrations. Results were obtained using primers targeting human-specific genomic sequence for qPCR assays with the PhoenixDx® ABC qPCR Mastermixes (Procomcure Biotech #PCCSKU12011) and cycled on Applied Biosystems™ QuantStudio 5 qPCR thermal cycler. Results confirm that the eluted gDNA is highly pure and free from inhibitors, optimal for qPCR.

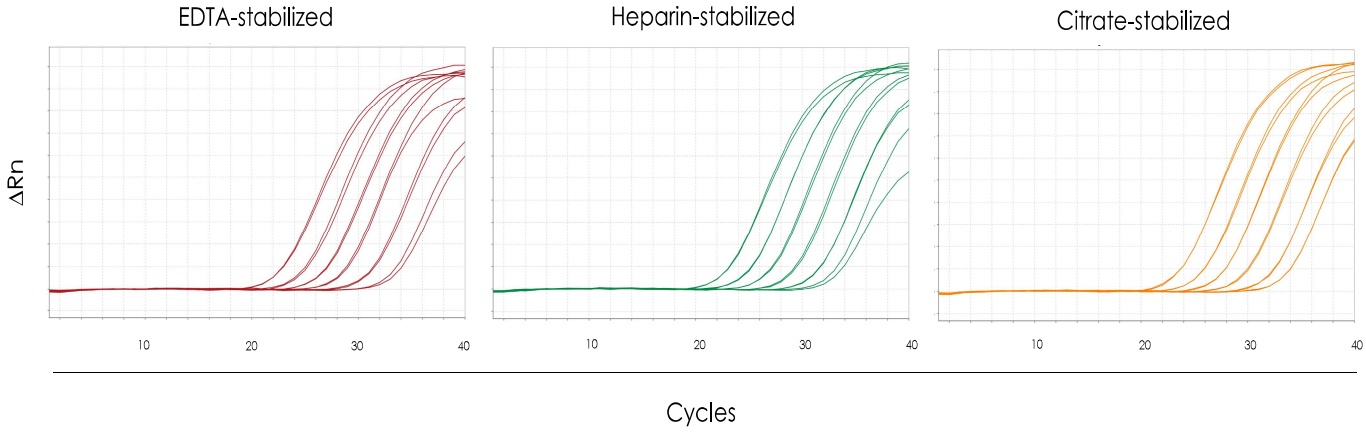
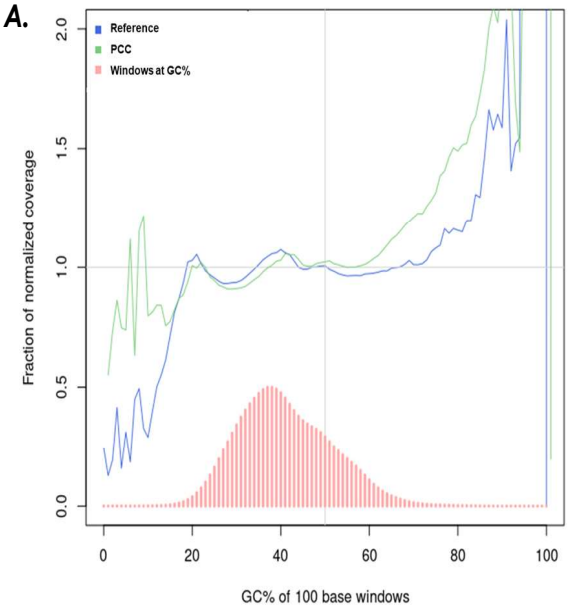


Figure 3. qPCR data using human-specific designed assays.

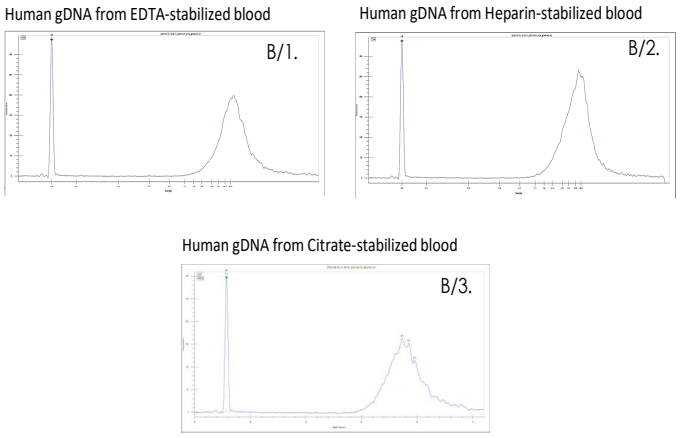
NGS Library Preparation

Libraries were prepared from 50 ng reference gDNA (**Reference**) and 50 ng human gDNA purified with the SphaeraMag® Genomic DNA Blood Purification Kit (**PCC**) using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina. Libraries were sequenced on an Illumina NovaSeq 6000. Reads were mapped using BWA and GC coverage was calculated using Picard's CollectGCBiasMetrics (v2.26.2). The gDNA was analyzed with the Genomic DNA Assay on a LabChip GX Touch (Perkin Elmer). The Genomic DNA Quality Score (GQS) represents the degree of degradation of a sample, with 5 corresponding to intact gDNA. Total gDNA yields for the representative samples were ~6 µg/200 µl whole blood. For purity assessment A260/A280 ratio was used. A ratio of ~1.8 is generally accepted as "pure" for gDNA.

A.



B.



C.

Sample description	GQS	Total yield (from 200µl blood)	A260/280
	[0-5]	ng	Ratio
Human gDNA from EDTA-stabilized blood	4.5	6560	1.88
Human gDNA from Heparin-stabilized blood	4.4	5680	1.91
Human gDNA from Citrate-stabilized blood	4.7	5700	1.94
Human gDNA Reference	3.8	-	1.96

Figure 4. A. GC bias plot. **B.** Electropherograms. **C.** Intactness, total yield and purity of the isolated gDNA compared to reference.

11. SAMPLE STORAGE AND HANDLING GUIDELINES

To ensure a high quality gDNA, fast processing of samples is highly recommended. If this is not possible, samples should be ideally stored according to the following recommendations.

- **Fresh blood samples** can remain stable for up to 7 days. DNA degradation occurs progressively in older samples resulting in loss of yield. Make sure that fresh blood samples are not older than a week and that they are stored properly (+4 to +8°C).

- **Frozen blood samples** should be kept frozen until processing, otherwise DNA degradation may occur. Avoid repeated freeze-thaw cycles.

12. PRE-APPLICATION PREPARATION

Make sure to get familiar with the complete extraction process before getting started.

GENERAL IMPORTANT NOTES BEFORE GETTING STARTED

- Prepare Wash Buffer I (only for Universal 96) by adding Isopropanol ≥99.5% (not supplied) as indicated on the bottle label and store at room temperature.
- Prepare Wash Buffer II (only for Universal 96) by adding Ethanol, absolute (not supplied) as indicated on the bottle label and store at room temperature.
- When using an alternative to a thermal mixer (e.g. heating block, incubator or water bath), vortex sample at a repetitive frequency for approx. 5 min (depending on the overall incubation time).
- Lysis/Binding Buffer, Wash Buffer I and Enhancer Solution may form precipitates when stored under cool conditions. Check individually bottled buffers for precipitation before use. Re-dissolve at +37°C and gently mix, if necessary.

13. ELUTION AND STORAGE

Buffer Composition
The Elution Buffer is 10 mM Tris-HCl, pH 8.0. Alternatively, nuclease-free water or other low ionic strength buffers can be used.

Temperature
Elution is strongly recommended with the Elution Buffer pre-heated to +60°C to improve elution efficiency.

Volume
The recommended elution volume ranges between 50-100 µl. A higher elution volume results in higher yield, but decreased gDNA concentration. A lower volume allows for higher concentration.

Storage
The eluted gDNA can be safely stored at +4 to +8°C for weeks to months. For long term storage, store the eluted gDNA at -80°C. Repeated freeze-thaw cycles should be avoided to maintain eluate integrity.

14. EXTRACTION PROTOCOLS

14.1. AUTOMATED: AUTO-PURE 96/PHOENIX-PURE 96

Compatible Kits: SphaeraMag® Genomic DNA Blood Purification Kit - Universal 96
SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled 96

Important:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled plates are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. If necessary, centrifuge the deep-well plates at 2,000 *xg* for 3 minutes to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Make sure to have V-bottom 96 deep-well plates containing reagents (Table 4) ready for use.

Step Name	Plate Position	Reagents	Volume per well
Loading	1	Tip comb	-
Digestion	2	Enhancer Solution	40 µl
-	3	-	-
Wash 1	4	Wash Buffer I	600 µl
Wash 2	5	Wash Buffer II	600 µl
Wash 3	6	Wash Buffer II	600 µl
-	7	-	-
Elution	8	Elution Buffer	100 µl

Table 4. Setup for V-bottom 96 deep-well plates compatible with Phoenix-Pure 96/Auto-Pure 96 device.

2. Remove sealing foil from pre-filled plates.
3. Transfer sample(s) and Proteinase K (**orange**) to appropriate well(s) (Table 5) on Position-2 Plate. Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

Step Name	Plate Position	Sample/Reagents	Volume per well
Loading	1	Tip comb	-
Digestion	2	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
-	3	-	-
Wash 1	4	Wash Buffer I	600 µl
Wash 2	5	Wash Buffer II	600 µl
Wash 3	6	Wash Buffer II	600 µl
-	7	-	-
Elution	8	Elution Buffer	100 µl

Table 5. Pipetting scheme for sample and Proteinase K transfer into V-bottom 96 deep-well plate prior to program 1 of 2.

4. Load V-bottom 96 deep-well plates onto instrument's plate positions according to plate layout (Table 5). Note: Make sure that all plates are placed in the correct orientation.
5. Set program 1 of 2 according to Table 6.

step	step name	plate	mix time (min)	mix amp (%)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	segment (1-5)	cycle time (1-10)	magnet speed (1-10)	1st segment time	2nd segment time
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Digest	2	20	80	0	300	3	55	2	1	3	15	15
3	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 6. Program setting for step 1 of 2 for "Genomic DNA Blood Purification" on Phoenix-Pure 96/Auto-Pure 96 device.

6. Run the program.
7. Once the program is finished, take the Position-2 Plate out of the device.
8. Mix the Lysis/Binding Buffer with Magnetic Beads in a nuclease-free tube for required amount of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads.
   Note: Ensure that beads stay fully mixed by constantly inverting the tube.
9. Transfer 240 µl Lysis/Binding Buffer and Magnetic Bead mix to each used well on the Position-2 Plate.
10. Load Position-2 Plate back onto the device.
11. Set program 2 of 2 according to Table 7.

step	step name	plate	mix time (min)	mix amp (%)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	segment (1-5)	cycle time (1-10)	magnet speed (1-10)	1st segment time (sec)	2nd segment time (sec)
1	Load	1	0	1	0	5	1	0	1	1	1	1	1
2	Lysis/Bind	2	5	80	0	500	3	37	2	1	4	20	20
3	Wash 1	4	1	80	0	600	5	0	2	1	3	20	20
4	Wash 2	5	1	80	0	600	5	0	2	1	3	20	20
5	Wash 3	6	2	80	2.5	600	5	0	2	1	3	20	20
6	Elution	8	1	80	0	100	3	60	2	2	3	30	30
7	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 7. Program setting for step 2 of 2 for "Genomic DNA Blood Purification" on Phoenix-Pure 96/Auto-Pure 96 device.

12. Run the program.
13. After the run is finished, remove V-bottom 96 deep-well plates and the tip comb. Transfer eluates from Position-8 plate (Elution Buffer) into new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

14.2. AUTOMATED: AUTO-PURE 32/PHOENIX-PURE 32

Compatible Kits: SphaeraMag® Genomic DNA Blood Purification Kit - Universal 96
SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled 32

Important:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled plates are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. If necessary, centrifuge the deep-well plate(s) at 2,000 *xg* for 3 minutes to remove droplets from the seal.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

1. Make sure to have U-bottom 96 deep-well plate(s) containing reagents (Table 8) ready to use.

Well	Reagents	Volume per well
Column 1/7	Enhancer Solution	40 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 8. Setup for an U-bottom 96 deep-well plate compatible with Phoenix-Pure 32/Auto-Pure 32 device.

2. Remove sealing foil from pre-filled plates.
3. Transfer sample(s) and Proteinase K (**orange**) to appropriate well(s) in columns 1/7 (Table 9).
Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

Well	Samples/Reagents	Volume per well
Column 1/7	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 9. Pipetting scheme for sample and Proteinase K transfer into U-bottom 96 deep-well plates prior to program 1 of 2.

4. Load U-bottom 96 deep-well plate(s) onto the instrument. Note: Make sure that each plate(s) is placed in the correct orientation.
5. Place new clean tip comb(s) in the instrument.
6. Set program 1 of 2 according to Table 10.

step	step name	well	mix time (min)	magnet (sec)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	mix pos (%)	mix amp (%)	magnet pos (%)	magnet speed (1-10)
1	Digest	1	20	68	0	300	7	55	0	80	0	3

Table 10. Program setting for step 1 of 2 for "Genomic DNA Blood Purification" on Phoenix-Pure 32/Auto-Pure 32 device.

7. Run the program.
8. Once the run is finished, leave tip comb(s) in the device and take the U-bottom 96 deep-well plate(s) out of the device.
9. Mix the Lysis/Binding Buffer with Magnetic Beads in a nuclease-free tube for required amount of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads.
   Note: Ensure that beads stay fully mixed by constantly inverting the tube.
10. Transfer 240 µl Lysis/Binding Buffer and Magnetic Bead mix to appropriate wells in column(s) 1/7.
11. Load the U-bottom 96 deep-well plate(s) back onto the device.
12. Set program 2 of 2 according to Table 11.

step	step name	well	mix time (min)	magnet (sec)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	mix pos (%)	mix amp (%)	magnet pos (%)	magnet speed (1-10)
1	Lysis/Bind	1	5	68	0	500	4	37	0	80	0	4
2	Wash 1	3	1	68	0	600	5	0	0	80	0	3
3	Wash 2	4	1	68	0	600	5	0	0	80	0	3
4	Wash 3	5	2	68	2.5	600	5	0	0	80	0	3
5	Elution	6	1	180	0	100	6	60	0	80	0	3
6	Waste	1	0.1	0	0	500	1	0	0	80	0	3

Table 11. Program setting for step 2 of 2 for "Genomic DNA Blood Purification" on Phoenix-Pure 32/Auto-Pure 32 device.

13. Run the program.
14. After the run is finished, remove the U-bottom 96 deep-well plate(s) and tip comb(s). Transfer the eluate(s) from the column(s) 6/12 to new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

14.3. AUTOMATED PURIFICATION: AUTO-PURE MINI

Compatible Kit: SphaeraMag® Genomic DNA Blood Purification Kit - Universal 96
SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled Mini

Important:

- Vortex Magnetic Beads thoroughly for 30 sec to fully resuspend the beads before each use.
- Tip combs and pre-filled cartridges are single use only. Do not re-use them.
- Make sure that solutions are at the bottom of each well and not on the seal. When using a V-bottom 96 deep-well plate, centrifuge the deep-well plate at 2,000 xg for 3 minutes to remove droplets from the seal, if necessary.
- The total volume in each well should not exceed 900 µl to avoid spilling and cross-contamination.

Samples can be processed **(a)** in cartridges (1 cartridge per sample) or **(b)** in a V-bottom 96 deep-well plate (16 samples per plate). Make sure to have reagents dispensed according to Table 12 or Table 13.

(a) Cartridge format:

Well	Reagents	Volume per well
Position 1	Enhancer Solution	40 µl
Position 2	-	-
Position 3	Wash Buffer I	600 µl
Position 4	Wash Buffer II	600 µl
Position 5	Wash Buffer II	600 µl
Position 6	Elution Buffer	100 µl

Table 12. Setup for a cartridge compatible with Auto-Pure Mini.

(b) V-bottom 96 deep-well format:

Well	Reagents	Volume per well
Column 1/7	Enhancer Solution	40 µl
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 13. Setup for a V-bottom 96 deep-well plate compatible with Auto-Pure Mini.

1. Remove sealing foil from cartridge(s) or V-bottom 96 deep-well plate.
2. Transfer sample(s) and Proteinase K (**orange**) to appropriate well(s) according to Table 14 or Table 15.
   Note: Do not pre-mix the Enhancer Solution and Proteinase K due to potential enzyme inactivation.

(a) Cartridge format:

Well	Samples/Reagents	Volume per well
Position 1	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
Position 2	-	-
Position 3	Wash Buffer I	600 µl
Position 4	Wash Buffer II	600 µl
Position 5	Wash Buffer II	600 µl
Position 6	Elution Buffer	100 µl

Table 14. Pipetting scheme for sample and Proteinase K transfer into cartridge(s) prior to program 1 of 2

(b) V-bottom 96 deep-well format:

Well	Samples/Reagents	Volume per well
Column 1/7	Enhancer Solution **Sample** **Proteinase K**	40 µl **200 µl** **20 µl**
Column 2/8	-	-
Column 3/9	Wash Buffer I	600 µl
Column 4/10	Wash Buffer II	600 µl
Column 5/11	Wash Buffer II	600 µl
Column 6/12	Elution Buffer	100 µl

Table 15. Pipetting scheme for sample and Proteinase K transfer into a V-bottom 96 deep-well plate prior to program 1 of 2.

3. Load cartridge(s) or V-bottom 96 deep-well plate onto instrument. Note: Make sure that cartridge(s) or V-bottom 96 deep-well plate are placed in the correct orientation.
4. Place new clean tip comb(s) in the instrument.
5. Set program according to Table 16.

step	step name	hole site	volume (µl)	mix time (min)	mix speed	dry time (min)	temperature (°C)	segments	every time (sec)	magnetization time (sec)	cycle	magnet speed (mm/sec)	mix scope (%)	mix pos.	magnet pos.
1	Digest	1	300	20	8	0	55	2	30	0	1	3	80	0	0
2	Waste	1	500	0.1	5	0	0	1	0	0	1	2.5	80	0	0

Table 16. Program setting for step 1 of 2 for "Genomic DNA Blood Purification" on Auto-Pure Mini device.

6. Run the program.
7. Once the run is finished, leave tip comb(s) in device and take the cartridge(s) or the V-bottom 96 deep-well plate out of the device.

8. Mix the Lysis/Binding Buffer with Magnetic Beads in a nuclease-free tube for required amount of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads.
   Note: Ensure that beads stay fully mixed by constantly inverting the tube.
9. Transfer 240 µl Lysis/Binding Buffer and Magnetic Bead mix to appropriate wells:
   (a) Cartridge format: Position 1
   (b) V-bottom 96 deep-well plate format: Column 1/7
10. Load cartridge(s) or the V-bottom 96 deep-well plate back onto the device.
11. Set program 2 of 2 according to Table 17.

step	step name	well	mix time (min)	magnet (sec)	wait time (min)	volume (µl)	mix speed (1-10)	temp (°C)	mix pos (%)	mix amp (%)	magnet pos (%)	magnet speed (1-10)
1	Lysis/Bind	1	5	68	0	500	4	37	0	80	0	4
2	Wash 1	3	1	68	0	600	5	0	0	80	0	3
3	Wash 2	4	1	68	0	600	5	0	0	80	0	3
4	Wash 3	5	2	68	2.5	600	5	0	0	80	0	3
5	Elution	6	1	180	0	100	6	60	0	80	0	3
6	Waste	1	0.1	0	0	500	1	0	0	80	0	3

Table 17. Program setting for step 2 of 2 for "Genomic DNA Blood Purification" on Auto-Pure Mini device.

12. Run the program.
13. After the run is finished, remove cartridge(s) or the V-bottom 96 deep-well plate and tip comb(s). Transfer the eluate from position 6 or column(s) 6/12 to new tubes, if required. Process eluates directly or store at +4 to +8°C for short-term or at -80°C for long-term storage.

14.4. MANUAL EXTRACTION

Preparation:

- Pre-heat appropriate volume of Elution Buffer to +60°C.

1. Transfer 40 µl Enhancer Solution to a nuclease-free 1.5 ml or 2 ml microcentrifuge tube.
2. Add 200 µl blood sample and 20 µl Proteinase K to the tube. Mix by pipetting up and down at least 5 times.
3. Incubate in a thermal mixer at +55°C with agitation at full speed (~1,400 *rpm*) for 20 min.
   Alternative: Incubate in a heating device at +55°C for 20 min. Invert the tube ~5 times during incubation.
4. Vortex Magnetic Beads thoroughly. Combine them with the Lysis/Binding Buffer in a nuclease-free tube for required amount of reactions. For each sample: Mix 200 µl Lysis/Binding Buffer + 40 µl Magnetic Beads. Note: Ensure that beads stay fully mixed by constantly inverting the tube.
5. Pipette 240 µl Lysis/Binding Buffer and Magnetic Bead mix to the processed sample.
6. Incubate in a thermal mixer at +37°C with agitation at full speed (~1,400 *rpm*) for 5 min.
   Alternative: Incubate in a heating device at +37°C for 5 min. Invert the tube 2 times during incubation.
7. Place the tube on a magnetic stand. Allow the magnetic beads to settle until the solution is completely clear.
8. Remove and discard the supernatant. Avoid disturbing the magnetic bead pellet.
9. Add 600 µl Wash Buffer I.
10. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
11. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
12. Remove and discard the supernatant. Avoid disturbing magnetic bead pellet.
13. Add 600 µl Wash Buffer II.
14. Remove the tube from the magnetic stand. Mix well by pipetting several times up and down.
15. Place the tube on the magnetic stand. Allow the magnetic beads to settle until the solution is completely clear.
16. Remove and discard the supernatant. Avoid disturbing magnetic bead pellet.
17. Repeat steps 13-16 for an additional washing step with Wash Buffer II.
18. Remove any residual liquid. Leave the tube open for 5 min to allow magnetic beads air dry.
19. Add 100 µl pre-heated Elution Buffer to the tube.
20. Remove the tube from the magnetic stand. Mix well by pipetting up and down ~10 times.
21. Incubate at room temperature for 1 min.
22. Place the tube on the magnetic stand. Allow the beads to settle until the solution is completely clear.
23. Transfer the eluate to a clean tube. Process directly, store at +4 to +8°C for short-term or at -80°C for long-term storage.

15. TROUBLESHOOTING GUIDE

Please use this guide to troubleshoot possible problems that may arise. For further assistance, please contact the technical support staff at support@procomcure.com.

Problem	Possible Cause	Solution
Low DNA Yield	Incomplete resuspension of SphaeraMag® Magnetic Beads	Thoroughly resuspend SphaeraMag® Magnetic Beads before use.
	DNA degradation during sample handling or storage	Process sample immediately after collection or removal from adequate storage.
	Incorrect preparation of Wash Buffers	Prepare Wash Buffers by adding the correct amount of Ethanol or Isopropanol according to instructions indicated on the bottle labels.
	Insufficient sample material	Increase lysis and binding time if the sample is diluted.
	Frozen blood samples not mixed properly after thawing	Thaw frozen blood on ice and mix the blood by gently inverting the tube.
Problems with downstream applications	Poor DNA quality	Do not freeze-thaw the isolated gDNA more than once or store at room temperature.
	Insufficient DNA was used	Quantify the isolated gDNA accurately and use sufficient gDNA.
	Ethanol carry-over	Dry the SphaeraMag® Magnetic Beads completely before adding Elution Buffer.
Carry-over of Magnetic Beads	Incomplete magnetization	Place the eluted samples on a magnetic stand device for additional 5 minutes or centrifuge at >4,000 xg for 5 minutes. When using automated isolation, check the instrument settings and increase bead binding time, if necessary.

Table 18. Troubleshooting guide.

16. CONSUMABLES AND RELATED PRODUCTS

Product	Content	Cat.No.
SphaeraMag® Genomic DNA Blood Purification Kit - Universal 96	96 preps	PCCSKU16067
SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled 96	96 preps for Phoenix-Pure 96 & Auto-Pure 96	PCCSKU16068
SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled 32	80 preps for Phoenix-Pure 32 & Auto-Pure 32	PCCSKU16069
SphaeraMag® Genomic DNA Blood Purification Kit - Pre-filled Mini	48 preps for Auto-Pure Mini	PCCSKU16070
Tip Combs for Auto-Pure 96 & Phoenix-Pure 96	2 pcs	PCCSKU30014SA
Tip Combs for Auto-Pure 32, Phoenix-Pure 32 & Auto-Pure Mini	20 pcs	PCCSKU16013
U-Bottom Deep-well Plates for Auto-Pure 32 & Phoenix-Pure 32	20 pcs	PCCSKU16014
V-Bottom Deep-well Plates for Auto-Pure 96, Phoenix-Pure 96 & Auto-Pure Mini	50 pcs	PCCSKU30002

Table 19. Consumables and related products.

Ordering information

For ordering SphaeraMag® Genomic DNA Blood Purification Kit and other products, visit us at

www.procomcure.com

or order via E-mail:

sales@procomcure.com

PRO COM CURE

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Quality Management System Certified
ISO 9001:2015
EN ISO 13485:2016