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LAB SYSTEMS

SphaeraMag® Genomic DNA Blood Purification Kit

Nucleic Acid Extraction for Manual and Automated Systems

Instruction Manual

Version 1.5

INTRODUCTION

The SphaeraMag® Genomic DNA Blood Purification Kit is designed for rapid and reliable isolation of genomic DNA from blood samples (compatible with anticoagulants EDTA, Heparin, Citrate). Isolated DNA can be further analyzed either for diagnostic purposes (e.g. pathogen detection, sequencing) or research applications (e.g. PCR analysis).

Samples are lysed in specifically formulated buffers to release nucleic acids. Genomic DNA is bound to the surface of paramagnetic beads, while proteins and cellular debris are removed during washing steps. The genomic DNA is then eluted in the provided Elution buffer. Alternatively, nuclease-free water or other low ionic strength buffer can be used. The procedure can be performed using automated systems (like Auto-Pure) or manually.

To counteract the co-isolation of RNA, an optional RNase A digest can be performed.

	SphaeraMag® Genomic DNA Blood PurificationKit - 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
Preps	96	96	80	48
SphaeraMag® RBC Buffer	75 ml	pre-filled	pre-filled	pre-filled
SphaeraMag® PBNC Buffer	75 ml	pre-filled	pre-filled	pre-filled
SphaeraMag® Wash Buffer	50 ml	pre-filled	pre-filled	pre-filled
SphaeraMag® Magnetic Beads	2 x 1 ml	2 x 1 ml	2 x 1 ml	pre-filled
SphaeraMag® gDNA Elution Buffer	10 ml	pre-filled	pre-filled	pre-filled
SphaeraMag® Proteinase K	1 ml	1 ml	1 ml	1 ml
SphaeraMag® RNase A	320 µl	320 µl	320 µl	320 µl

Table 1. Content of SphaeraMag® Genomic DNA Blood Purification kit.

SphaeraMag® RBC Buffer and SphaeraMag® PBNC Buffer contain chaotropic salts. Please handle with appropriate laboratory safety measures.

STORAGE AND STABILITY

- Upon arrival, SphaeraMag® Magnetic Beads must be stored at +2 °C to +8 °C.
- Upon arrival, SphaeraMag® Proteinase K and SphaeraMag® RNase A must be stored at -15 °C to -25 °C.
- The expiry dates of the kit and single components are stated on the outer packaging and each individual reagent.

ADDITIONAL MATERIALS REQUIRED

- Ethanol, absolute (SphaeraMag® Universal)
- Suitable reagent dispensing options
- Consumables for isolation devices for automated extraction
- Magnetic separation rack and thermal mixer for 1.5 ml or 2 ml tubes (or alternative compatible heating device) for manual purification

PRE-APPLICATION PREPARATION

- Add absolute ethanol to the SphaeraMag® Wash Buffer as stated on the bottle (only for SphaeraMag® Universal). Store at room temperature.

- For automated isolation, pipet the reagents into the appropriate plate(s), according to respective protocol used.

KIT COMPATIBILITY

- Hamilton Microlab® STAR / MagEx STARlet / NIMBUS
- KingFisher™ BioSprint®
- Tecan Dream Prep™ NAP
- Phoenix-Pure-96 / Phoenix-Pure-32
- Allsheng Auto-Pure-96 / Auto-Pure-32 / Auto-Pure Mini

AUTOMATED PURIFICATION: AUTO-PURE-96 / PHOENIX-PURE-96

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

Important:

- Resuspend SphaeraMag® Magnetic Beads thoroughly for 30 sec before use.
- The total volume in each well should not exceed 920 µl to avoid spilling and cross-contamination.

1. Take six V-bottom 96 deep-well plates. When using pre-filled plates, carefully remove the sealing foil. Add samples, Magnetic Beads, Proteinase K and RNase A (optional) to appropriate wells according to Table 2.

Step	Plate Position	Samples/Reagents	Volume
Loading	1	Tip comb	/
Lysis	2	SphaeraMag® PBNC Buffer **SphaeraMag® Proteinase K** **SphaeraMag® RNase A (optional)**	700 µl **10 µl** **3 µl**
Binding	3	SphaeraMag® RBC Buffer **Sample** **SphaeraMag® Magnetic Beads**	700 µl **max. 200 µl** **20 µl**
-	4	-	-
Wash 1	5	SphaeraMag® Wash Buffer	600 µl
Wash 2	6	SphaeraMag® Wash Buffer	600 µl
-	7	-	-
Elution	8	SphaeraMag® gDNA Elution Buffer	100 µl

Table 2. Setup of 96 deep-well plates and reagent volumes compatible with Auto-Pure-96.

2. Start the instrument. Place the deep-well plates on the corresponding positions into the instrument. Note: Make sure that all plates are placed in the correct orientation.

3. Set the program according to Table 3.

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	Bind	3	1	80	0	900	2	0	2	1	3	10	10
3	Lysis	2	5	80	0	700	5	65	2	1	3	10	10
4	Wash 1	5	1	80	0	600	5	0	2	1	3	5	5
5	Wash 2	6	1	80	10	600	5	0	2	1	3	5	5
6	Elution	8	5	80	0	100	3	40	1	3	3	30	1
7	Unload	1	0	1	0	5	1	0	1	1	1	1	1

Table 3. Program setting for "Genomic DNA Blood Purification" on Auto-Pure-96.

4. Run the program.

5. After the run is finished, remove plates and tip comb. Transfer eluates from the elution plate into new tubes, if required. Process directly, store at 4 °C to 8 °C for short-term or at -80 °C for long-term storage.

AUTOMATED PURIFICATION: AUTO-PURE-32 / PHOENIX-PURE-32

Compatible Kit: SphaeraMag® Genomic DNA Blood Purification Kit - pre-filled 32

Important:

- Resuspend SphaeraMag® Magnetic Beads thoroughly for 30 sec before use.
- The total volume in each well should not exceed 920 µl to avoid spilling and cross-contamination.

1. Take a U-bottom 96 deep-well plate. When using pre-filled plates, carefully remove the sealing foil. Add samples, Magnetic Beads, Proteinase K and RNase A (optional) to appropriate wells according to Table 4.

Well	Samples/Reagents	Volume
Column 1/7	SphaeraMag® PBNC Buffer **SphaeraMag® Proteinase K** **SphaeraMag® RNase A (optional)**	700 µl **10 µl** **3 µl**
Column 2/8	SphaeraMag® RBC Buffer **Sample** **SphaeraMag® Magnetic Beads**	700 µl **max. 200 µl** **20 µl**
Column 3/9	-	-
Column 4/10	SphaeraMag® Wash Buffer	600 µl
Column 5/11	SphaeraMag® Wash Buffer	600 µl
Column 6/12	SphaeraMag® gDNA Elution Buffer	100 µl

Table 4. Setup of 96 deep-well plate and reagent volumes compatible with Auto-Pure-32.

2. Start the instrument. Place new clean tip comb(s) in the instrument (re-use of tip combs will cause cross-contamination). Place the 96-well plate containing sample(s) and reagents into the instrument.

3. Set the program according to Table 5.

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	Bind	2	1	60	0	900	2	0	0	80	0	3
2	Lysis	1	5	60	0	700	5	65	0	80	0	3
3	Wash 1	4	1	45	0	600	5	0	0	80	0	3
4	Wash 2	5	1	45	10	600	5	0	0	80	0	3
5	Elution	6	5	90	0	100	3	40	0	80	0	3
6	Waste	1	0.1	0	0	700	1	0	0	80	0	3

Table 5. Program setting for "Genomic DNA Blood Purification" on Auto-Pure-32.

4. Run the program.

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

AUTOMATED PURIFICATION: AUTO-PURE MINI

Compatible Kit: SphaeraMag® Genomic DNA Blood Purification Kit - pre-filled Mini

Important:

- The total volume in each well should not exceed 920 µl to avoid spilling and cross-contamination.

1. Take required amount of extraction cartridges (1 per sample). Carefully remove the sealing foil. Add sample, Proteinase K and RNase A (optional) to appropriate wells according to Table 6.

Well	Samples/Reagents	Volume
Position 1	SphaeraMag® PBNC Buffer **SphaeraMag® Proteinase K** **SphaeraMag® RNase A(optional)**	700 µl **10 µl** **3 µl**
Position 2	SphaeraMag® RBC Buffer **Sample**	700 µl **max. 200 µl**
Position 3	SphaeraMag® Magnetic Beads	320 µl
Position 4	SphaeraMag® Wash Buffer	600 µl
Position 5	SphaeraMag® Wash Buffer	600 µl
Position 6	SphaeraMag® gDNA Elution Buffer	100 µl

Table 6. Setup of extraction cartridge and reagent volumes compatible with Auto-Pure Mini.

2. Start the instrument. Place new clean tip comb(s) in the instrument (re-use of tip combs will cause cross-contamination). Place extraction cartridge containing the sample and reagent into the instrument.

3. Set the program according to Table 7.

step	step name	hole site	volume (µl)	mix time (min)	mix speed	dry time (min)	temperature (°C)	segments	every time (sec)	magnetization time (s)	cycle	magnet speed (mm/sec)	mix scope (%)	Mix pos.	Magnet pios.
1	Bead	3	320	1	8	0	-	2	30	0	1	1	80	0	0
2	Bind	2	900	1	2	0	-	2	30	0	1	3	80	0	0
3	Lysis	1	700	5	5	0	65	2	30	0	1	3	80	0	0
4	Wash 1	4	600	1	5	0	-	2	30	0	1	3	80	0	0
5	Wash 2	5	600	1	5	10	-	2	30	0	1	3	80	0	0
6	Elution	6	100	5	3	0	40	2	45	0	1	3	80	0	0
7	Waste	1	700	0.1	1	-	-	1	0	0	0	2.5	80	0	0

Table 7. Program setting for "Genomic DNA Blood Purification" on Auto-Pure Mini device.

4. Run the program.

5. After the run is finished, remove extraction cartridge(s) and tip comb(s). Transfer the eluate from position 6 to a new tube if required. Process directly, store at 4 °C to 8 °C for short-term or at -80 °C for long-term storage.

MANUAL PURIFICATION

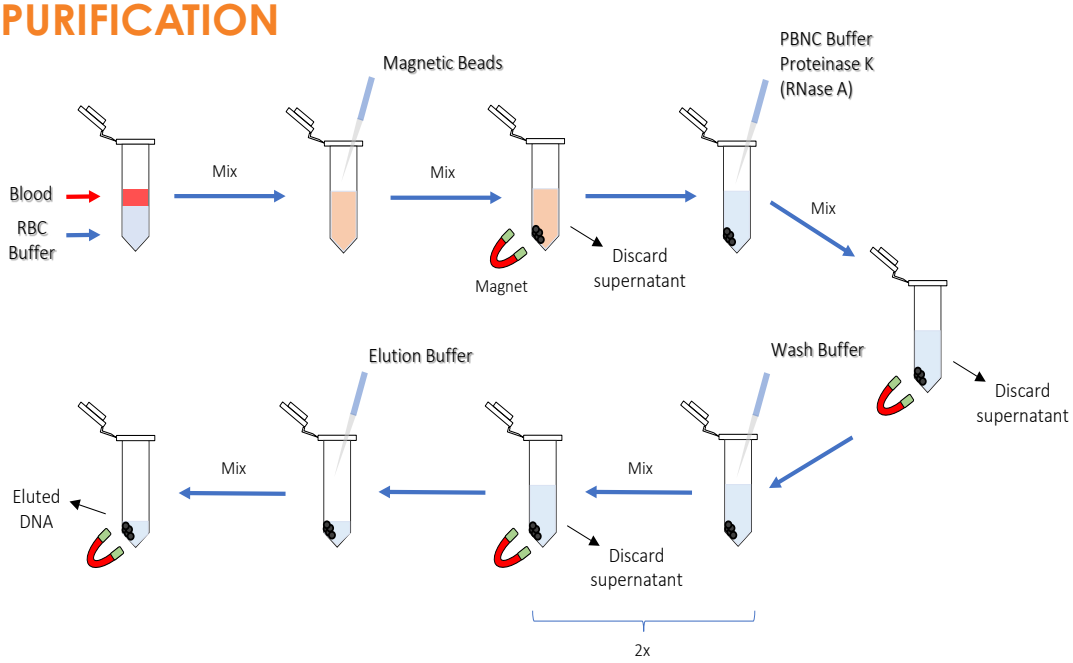

Figure 1. Illustrated purification protocol.

Preparation:

- Pre-heat appropriate volume of SphaeraMag® gDNA Elution Buffer to +40 °C.

1. Transfer 700 µl RBC Buffer to a clean 1.5 ml or 2 ml microcentrifuge tube.
2. Add max. 200 µl of blood sample to the tube. Mix by pipetting up and down at least 5 times. Note: If processing less than 200 µl sample, bring volume up to 200 µl with nuclease-free water.
3. Thorougly resuspend Magnetic Beads by vigorous vortexing for 30 sec. Add 20 µl Magnetic Beads to each tube. Mix by pipetting up and down at least 10-15 times.
4. Place the tube on a magnetic stand. Allow the beads to settle until the solution is completely clear.
5. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
6. Remove the tube from the magnetic stand.
7. Add 700 µl PBNC Buffer, 10 µl Proteinase K and 3 µl RNase A (optional). Mix by pipetting up and down.
8. Incubate in a thermal mixer at +65 °C with agitation at full speed (~ 1,400 *rpm*) for 5 min. Alternative: Incubate in heating device at +65 °C for 5 min. Invert tubes 2 times during incubation.
9. Place tube on a magnetic stand. Allow the beads to settle until the solution is completely clear.
10. Remove and discard the supernatant. Avoid disturbing the Magnetic Bead pellet.
11. Remove the tube from the magnetic stand.
12. Add 600 µl Wash buffer. Mix well by pipetting several times up and down.
13. Place tube on a magnetic stand. Allow the beads to settle until the solution is completely clear.
14. Remove and discard the supernatant. Avoid disturbing Magnetic Bead pellet.
15. Repeat steps 12-14 for a second washing step.
16. Leave the tube open allowing Magnetic Beads to air dry for 5 min. Remove any residual liquid.
17. Remove tube from the magnetic stand.
18. Add 100 µl pre-heated gDNA Elution Buffer to the tube. Mix well by pipetting up and down ~10 times.
19. Incubate at room temperature for 1 min.
20. Place tube on a magnetic stand. Allow the beads to settle until the solution is completely clear.
21. Transfer the clear supernatant containing eluate to a clean tube. Process directly, store at 4 °C to 8 °C for short-term or at -80 °C for long term storage.

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 Yield	Incomplete resuspension of SphaeraMag® Magnetic Beads	Thoroughly resuspend SphaeraMag® Magnetic Beads before use.
	DNA degraded during sample handling /storage	Immediately process sample after collection or removal from storage.
	Wash Buffer not prepared correctly	Prepare Wash Buffer adding the correct amount of ethanol.
	Insufficient sample material	Increase lysis and binding time if the sample is diluted.
Problems with downstream applications	Poor DNA quality	Do not freeze / thaw the isolated DNA more than once or store at room temperature.
	Insufficient DNA was used	Quantify the isolated DNA accurately and use sufficient DNA.
	Ethanol carry-over	Dry the SphaeraMag® Magnetic Beads completely before adding elution buffer.
Carry-over of Magnetic Beads	SphaeraMag® Magnetic Beads did not fully magnetize on last step	Place the eluted samples on a magnetic stand device for additional 5 minutes or centrifuge at >4,000 x *g* for 5 minutes. When using automated isolation, check the instrument settings and increase bead binding time, if necessary.

CONSUMABLES & RELATED PRODUCTS

	Content	Cat.No.
SphaeraMag® Genomic DNA Blood Purification Kit - Universal 96	96 preps	TRAXSKU16067
SphaeraMag® Genomic DNA Blood Purification Kit - pre-filled 96	96 preps for Phoenix-Pure-96 & Auto-Pure-96	TRAXSKU16068
SphaeraMag® Genomic DNA Blood Purification Kit - pre-filled 32	80 preps for Phoenix-Pure-32 & Auto-Pure-32	TRAXSKU16069
SphaeraMag® Genomic DNA Blood Purification Kit - pre-filled Mini	80 preps for Auto-Pure-Mini	TRAXSKU16070
Tip Combs for AutoPure-96 & Phoenix-Pure-96	60 pcs	TRAXSKU30014
Magnetic Rods for AutoPure-32 & Phoenix-Pure-32	20 pcs	TRAXSKU16013
U-Bottom Plates for AutoPure-32 & Phoenix-Pure-32	20 deepwell plates	TRAXSKU16014
V-Bottom Plates for AutoPure 96, Phoenix-Pure-96 & AutoPure Mini	50 deepwell plates	TRAXSKU30002

Ordering information

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

at www.traxlabsystems.com

or order via E-mail:

sales@traxconnects.com

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