EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMAGNET Blood DNA Purification Kit

Kit for isolation of DNA from fresh and frozen blood samples.

Cat. no. E3429

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Table of Contents

Kit content	96 preps E3429-01	Storage/Stability
Proteinase K (20 mg/ml)	1.2 ml	-20°C
Lyse Blood	32 ml	15-25°C
Sol Blood	58 ml	15-25°C
Wash B1	46 ml	15-25°C
Wash B2	70 ml	15-25°C
Wash B3	70 ml	15-25°C
Elution	30 ml	15-25°C
Magnetic Beads	1 ml	2-8°C
Protocol	1	

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the isolation of total DNA (genomic, mitochondrial) from fresh or frozen blood samples treated with anticoagulants (EDTA-K2, EDTA-K3, sodium citrate or heparin) and from blood spots by silica-covered Magnetic Beads. The kit is designed for both a manual and an automatic use.

NOTE 2 · Sample Amount. Recommended sample amount is 200 µl. For sample volumes less than 200 µl, add PBS or 0.9% NaCl to adjust the volume to 200 µl.

NOTE 3 · Sample storage. Blood treated with anticoagulants can be kept in 4°C up to a few days or frozen in -20°C (up to 4 weeks) or -70°C. Samples can be frozen up to 3 times without the considerable decrease in DNA isolation efficiency or quality.

NOTE 4 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of Magnetic Beads and Proteinase K. Magnetic Beads should be kept at 2-8°C and Proteinase K at -20°C.

NOTE 5 · Maintaining Good Working Practice. All solutions should be kept tightly closed to avoid evaporation and resulting concentration changes of buffer components. To obtain high quality DNA, stick carefully to the protocol provided below.

NOTE 6 · Elution buffer is a low salt solution, that contains no metal ion chealators (e.g. EDTA) that can inhibit subsequent enzymatic reactions. Elution buffer composition is suitable for downstream applications such as digestion with restriction enzymes, phosphorylation, ligation, Sanger sequencing, NGS etc. It is also possible to elute the DNA with Tris-HCl, water or TE.

Equipment and reagents to be supplied by the user

- Magnetic stand E0361 for 16 tubes, E0362 for 24 tubes, E0363 for 96-well plate. To be purchased separately.
- Ethanol 96-100 %, disposable gloves, sterile pipette tips, sterile 1.5-2 ml tubes or 96-well plates with working volume at least 0.8 ml (for samples treated with EDTA or sodium citrate) or 1.0 ml (for samples treated with heparin), heating block capable of incubation at 65°C (for samples treated with heparin), vortex.

Protocol

1. To 1.5-2 ml tube add 10 µl **Proteinase K** and 200 µl blood.
   - *For sample volumes less than 200 µl, add PBS or 0.9% NaCl to adjust the volume to 200 µl.*
2. Add 80 µl **Lyse Blood**.
3. Mix by vortexing 30 s or pipetting 20 times.
4. Incubate for 20 min at room temperature, mix every 10 min.
   - *20 min is sufficient for sample digestion, but the time can be prolonged if needed.*
5. Add 500 µl **Sol Blood**. Mix by vortexing 30 s or pipetting 20 times.
6. Resuspend **Magnetic Beads** before removing them from the storage tube by vortexing or pipetting. Add 10 µl of resuspended **Magnetic Beads** to the sample and mix by vortexing or pipetting for 30 s. Incubate the sample at room temperature for 5 min.
   - *If working on 96-well plate format transfer the samples to the wells on the plate with working volume at least 0.8-1.0 ml.*
   - *If the sample volume is greater than the volume of the well or the magnet force is too low, transfer a part of the sample, place the plate on the magnet, after Magnetic Beads separation remove the supernatant. Continue the sample transfer to the 96-well plate until all the remaining part of the sample is processed.*
7. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets (3 min).
8. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 400 µl of **Wash B1** and mix by pipetting or vortexing for 10 s.
9. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
10. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash B2** and mix by pipetting or vortexing for 10 s (second washing with **Wash B1**).
11. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
12. Remove and discard the supernatant by pipetting.
13. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash B3** and mix by pipetting or vortexing for 10 s.
14. Remove and discard the supernatant by pipetting. Leave the open tubes/plate in magnetic stand and air dry the beads for 15 min.
    - *Wash B3 contains alcohol, make sure all the solution evaporates before proceding to the next step.*
15. Add 100-200 µl **Elution** to the tube/well and mix by pipetting or vortexing. Incubate for 5 min at room temperature.

16. Separate the **Magnetic Beads** against the side of the wells. After all the beads have been attached to the magnets transfer the supernatant containing the purified DNA to a suitable tube/plate. DNA is ready for analysis/manipulations. Isolated DNA can be stored either at 2-8° or at -20°.

Appendix 1. DNA isolation for samples treated with heparin

NOTE 1 • The protocol is recommended for PCR inhibitors removal for samples treated with anticoagulant heparin, but can be employed for EDTA or citrated treated samples alternativaly to the main protocol if needed.

NOTE 2 • During the procedure the use of heating block (65°C) is needed.

NOTE 3 • The working volume for 96-well plates of at least 1.0 ml is needed.

NOTE 4 • The use of 96-100 % ethanol is needed.

1. To 1.5-2 ml tube add 10 µl of **Proteinase K** and 200 µl blood.
   - *For sample volumes less than 200 µl, add PBS or 0.9% NaCl to adjust the volume to 200 µl.*
2. Add 300 µl **Lyse Blood**.
3. Mix by vortexing 30 s or pipetting 20 times.
4. Incubate 30 min at 65°C mixing each 10 min. After the incubation cool down the samples to room temperature.
5. Add 500 µl ethanol (96-100%). Mix by vortexing or pipetting.
6. Continue from step 6 of the main protocol.

Appendix 2. DNA isolation from blood spots

1. Cut off the fragment of spotted material/Whatman papier (do not exceed 1 cm^2). Cut the material into small pieces. Place the pieces in the 2 ml Eppendorf tube. Spots from solid surfaces should be scratched and poured into the 2 ml Eppendorf tube.
2. Add 200 µl PBS and leave the soaked material for 2 h.
3. Add 10 µl **Proteinase K** and continue from step 2 of the main protocol (page 4).

Safety Information

Proteinase K

Danger

H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
P261 Avoid breathing vapours/spray.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P342+**P311** If experiencing respiratory symptoms: call a poison center or doctor/ physician.

Lyse Blood

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P261 Avoid breathing vapours/spray.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+**P312** If swallowed: call a poison center/ doctor if you feel unwell.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P333+**P313** If skin irritation or rash occurs: get medical advice/attention.
P337+**P313** If eye irritation persists: get medical advice/ attention.
EU**H208** Contains ethylenediammonium dichloride. May produce an allergic reaction.

Sol Blood

Danger

H226 Flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+**P235** – Store in a well-ventilated place. Keep cool.

Wash B1

Danger

H226 Flammable liquid and vapour.
H302+**H332** Harmful if swallowed or if inhaled.
H315 Causes skin irritation.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+**P312** If swallowed: call a poison center/ doctor if you feel unwell.
P302+**P352** If on skin: wash with plenty of water.
P304+**P340** If inhaled: remove person to fresh air and keep comfortable for breathing.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Wash B2 / Wash B3

Danger

H225 Highly flammable liquid and vapour.
H319 Causes serious eye irritation.
P210 Keep away from heat, hot surfaces, sparks, open flames and other ignition sources. No smoking.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P403+**P235** Store in a well-ventilated place. Keep cool.
P337+**P313** If eye irritation persists: get medical advice/ attention.

- ***GeneMAGNET Blood DNA Purification Kit is designed for rapid manual or automatic purification of total DNA (genomic, mitochondrial) from fresh or frozen human blood treated with EDTA or sodium citrate anticoagulant using silica-covered magnetic beads.***

  The new lysis buffer is specially designed to efficiently lyse blood cells, release DNA from nucelic and bind to magnetic beads without contaminants. Traces of contaminants remaining in the solution are efficiently removed in three wash steps. High-quality cellular DNA is then eluted to the DNA storage buffer. Isolated DNA is ready for downstream applications without the need for ethanol precipitation.

- **GeneMAGNET line is based on the use of silica paramagnetic beads (Magnetic Beads) for selective binding of RNA and DNA. The use of specially designed binding and washing buffers enables the efficient purification of highly pure nucleic acids.**

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