EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Quick Blood DNA Purification Kit

Kit for quick isolation of DNA from fresh or frozen blood

Cat. no. E3565

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Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the rapid isolation of highly pure genomic DNA from whole blood, serum, plasma or other body fluids.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg and maximum volume of the column reservoir is 650 µl. One minicolumn enables purification of DNA from up to 200 µl blood/body fluids. Blood can be stored in the presence of anticoagulants at 2–8°C (up to several days) or frozen (prefered temperature -70°C).

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature with the exception of RNase A and Proteinase K. RNase A should be kept at 2–8°C and Proteinase K at -20°C.

NOTE 4 · 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.

Content	50 preps E3565-01	150 preps E3565-02	Storage/Stability
Buffer QB	1.8 ml	5.4 ml	15-25°C
RNase A (10 mg/ml)	0.12 ml	0.36 ml	2-8°C
Proteinase K (20 mg/ml)	0.6 ml	1.8 ml	–20°C
Sol QB	12 ml	36 ml	15-25°C
Wash QBX1	30 ml	90 ml	15-25°C
Wash QBX2	30 ml	90 ml	15-25°C
Elution	18 ml	54 ml	15-25°C
DNA Binding Columns	50	3 x 50	15-25°C
Protocol	1	1	

Equipment and reagents to be supplied by the experimenter

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, ethanol 96–100%, a heating block capable of incubation at 70°C.
- The kit does not contain PBS. To prepare sterile PBS, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO_4 in 800 ml H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 liter.

Protocol

1. Apply 30 µl of activation **Buffer QB** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column (for best results at least 10 min).

   - *Addition of Buffer QB onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.*

   - *The membrane activation should be done before starting isolation procedure.*

2. Add 200 µl blood/body fluid to 1.5–2 ml Eppendorf tube.

   - *For sample volumes less than 200 µl, add PBS to adjust the volume to 200 µl.*

   - *If RNA-free DNA is crucial for downstream applications, add 2 µl RNase A. Mix by vortexing and incubate 5 min at room temperature.*

   - *If purifying DNA viruses, it is recommended to start with 200 µl serum or plasma to prepare pure viral DNA (cellular DNA-free).*

3. Add 10 µl of **Proteinase K** and next 200 µl of **Sol QB** buffer. Mix thoroughly by vortexing.

4. Incubate for 10 min at 70°C.

5. Add 200 µl of ethanol (96–100%). Mix thoroughly by vortexing.

6. Centrifuge for 1 min at 11 000 x g.

7. Transfer the lysate to the **DNA binding spin-column**, placed in the collection tube.

8. Centrifuge for 1 min at 11 000 x g. Remove the spin-column, pour off supernatant and place back into the receiver tube.

   - *Continue centrifugation, if not all of the lysate passed through the column.*

9. Add 500 µl of **Wash QBX1** buffer and spin down at 11 000 x g for 1 min.

10. Remove spin-column, pour off supernatant, replace back spin-column.

11. Add 500 µl of **Wash QBX2** buffer and spin down at 11 000 x g for 1 min.

12. Remove spin-column, pour off supernatant, replace spin-column.

13. Spin down at 11 000 x g for 1 min to remove traces of the **Wash QBX2** buffer.

14. Place spin-column into new receiver tube (1.5–2 ml) and add 50–150 µl of **Elution** buffer to elute bound DNA.

    - *Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transferring traces of DNA between the spin-columns do not touch the spin-column walls with the micro-pipette.*

    - *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*

15. Incubate spin-column/receiver tube assembly for 2 min at room temperature.

16. Spin down at 11 000 x g for 1 min.

17. Remove spin column, cap the receiver tube. DNA is ready for analysis/manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

Safety Information

Buffer QB

Danger

H314 Causes severe skin burns and eye damage.
P280 Wear protective gloves/protective clothing/eye protection/face protection.
P301+P330+P331 If swallowed: Rinse mouth. Do not induce vomiting.
P303+P361+P353 If on skin (or hair): take off immediately all contaminated clothing. Rinse skin with water [or shower].
P305+P351+P338 If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 Immediately call a poison center/doctor.
P405 Store locked up.

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.

Sol QB

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.
EUH208 Contains ethylenediammonium dichloride. May produce an allergic reaction.

Wash QBX1

Warning

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.
przypadku utrzymywania się działania drażniącego na oczy: zasięgnąć porady/ zgłosić się pod opiekę lekarza.
P403+P235 Przechowywać w dobrze wentylowanym miejscu. Przechowywać w chłodnym miejscu.

Wash QBX2

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.

GeneMATRIX is synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures.

Novel binding and washing buffers are developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids. Matrix is conveniently pre-packed in ready-to-use spin-format. Unique chemical composition of the matrixes along with optimized construction of spin-columns improve the quality of final DNA or RNA preparation. To speed up and simplify isolation procedure, the key buffers are colour coded, which allows monitoring of complete solution mixing and makes purification procedure more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Such DNA or RNA can be directly used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybrydization among others. Additional advantage is reproducibility of matrix performance, as component preparation is carried at Eurx Ltd.

GeneMATRIX Quick Blood DNA Purification Kit is designed for the rapid isolation of highly pure genomic DNA from fresh or frozen blood, serum, plasma or other body fluids. It is also possible to purify viral DNA from blood samples. Purified DNA is free of contaminants, such as: RNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.

Blood/body fluid sample is lysed in the presence of special buffer containing large amounts of chaotropic ions and Proteinase K. Proteinase K digests cellular proteins, including stripping-off DNA of all bound proteins, among them nucleases. Appropriate conditions for binding of DNA to the GeneMATRIX resin is created by addition of ethanol to the lysate. During brief centrifugation step DNA binds to the silica membrane in the spin-column, while contaminants pass through. Traces of contaminants remaining on the resin are efficiently removed in two wash steps. High-quality cellular DNA is then eluted in low salt buffer, e.g.: Tris-HCl, TE or water. Isolated DNA is ready for downstream applications without the need for ethanol precipitation.

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