EURx Molecular Biology Products

# GeneMATRIX Quick Blood DNA Purification Kit

Kit for quick isolation of DNA from fresh or frozen blood

Cat. no. E3565

**Version 1.2**

**January, 2008**

For laboratory use only.
Not for drug, household or other uses.

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**Note 1:** The kit is designed for the rapid isolation of highly pure genomic DNA from whole blood, serum, plasma or other body fluids.
**Note 2:** 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:** 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:** All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
**Note 5:** The kit does not contain 96 % ethanol and 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 40 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.

**Note 1:** Addition of Buffer QB onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.
**Note 2:** The membrane activation should be done before starting isolation procedure.

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

**Note 1:** For sample volumes less than 200 µl, add PBS to adjust the volume to 200 µl.
**Note 2:** If RNA-free DNA is crucial for downstream applications, add 2 µl **RNase A**. Mix by vortexing and incubate 5 min at room temperature.
**Note 3:** 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 **Proteinase K** and next 200 µl **Sol QB** buffer.

4. Vortex the mixture thoroughly.

5. Incubate for 10 min at 70°C.

6. Add 200 µl **96 % ethanol**.

7. Vortex the mixture thoroughly.

8. Centrifuge for 1 min at 12000 rpm.

9. Transfer the lysate to the spin-column, placed in the collection tube.

10. Centrifuge for 2 min at 12000 rpm.

**Note 1:** Continue centrifugation, if not all of the lysate passed through the column.

11. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

12. Add 500 µl **Wash QBX1** buffer to the spin-column and centrifuge for 1 min at 12000 rpm.

13. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

14. Add 500 µl **Wash QBX2** buffer to the spin-column and centrifuge for 2 min at 12000 rpm.

15. Place the spin-column in a new collection tube (1.5-2 ml) and add 50-200 µl of **Elution** buffer (10 mM Tris-HCl, pH 8.5) heated to 70°C to elute bound DNA.

**Note 1:** Addition of the elution buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.

**Note 2:** The following elution solutions can be used:

1. 5-10 mM Tris-HCl buffer, pH 8.0-9.0
2. 0.5-1 x TE buffer, pH 8.0-9.0 (not recommended for DNA sequencing).
3. Other special application buffers can be used, if their pH and salt concentration is similar to that of 5-10 mM Tris-HCl, pH 8.0-9.0.

16. Incubate the spin-column/collection tube assembly for 3 min at room temperature.

17. Centrifuge the spin-column for 1 min at 12000 rpm.

**Optional:**

18. Repeat elution once again as described in steps 15-17.

**Note 1:** This step improves DNA recovery from the column. A new collection tube can be used to prevent dilution of the first eluate or collection tube from step 15 can be reused to combine the eluates.

**Note 2:** More than 200 µl should not be used to elute into a single 1.5 ml microcentrifuge tube, as the spin-column will come into contact with the eluate, causing DNA contamination.

19. Discard the spin-column, cap the collection tube. DNA is ready for analysis/manipulation. It can be stored either at 2÷8°C or at -20°C.

**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.