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# GeneMATRIX Human Blood RNA Purification Kit

Kit for isolation of total RNA from fresh human blood.

Cat. no. E3596

**Version 1.0**

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**Note:** Store homogenization and RNA binding spin-columns at 2÷8°C.

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

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**Note 1:** This kit is designed for isolation of total RNA (longer than 200 bases) from fresh human blood. **Kit is not suitable for isolation of RNA from frozen blood.**

**Note 2:** Blood must not be frozen. It is possible to store blood samples at 4°C for up to a few hours. Further extending the storage time leads to degradation of RNA and to gene induction. Both in vitro RNA degradation and gene induction can lead to an under- or overestimation of in vivo relative gene transcript number. It is possible to use Heparin or Citrate- or EDTA- stabilized blood samples.

**Note 3:** One mini columns allows purification of RNA with no more than 1.5 ml of blood.

**Note 4:** The total RNA binding capacity is 100 µg per spin-column.

**Note 5:** Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

**Note 6:** After freezing, the blood is not suitable for isolation of RNA using the kit.

**Note 7:** **Lyse RBC** buffer is supplied as a 5x concentrated. Please add the appropriate amount of RNase-free water (see label on the buffer) before using it.

**Note 8:** Add 10 µl β-mercaptoethanol (β-ME) per 1 ml buffer RL before use. Buffer RL is stable for 1 month after addition of β-ME.

**Note 9:** All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. Store the components of the kit at 2÷8°C.

**Note 10:** To obtain RNA of the highest purity it is important to follow the protocol provided below carefully. During the procedure, work quickly. All steps should be performed at room temperature.

Equipment and reagents to be supplied by user: β-mercaptoethanol (14.3 M, β-ME), ethanol 96-100%, microcentrifuge, disposable gloves, sterile RNase-free pipet tips, sterile RNase-free 1.5-2 ml tubes. When the blood volume exceeds 400 µl – appropriate size plastic tubes for erythrocytes lysis and centrifugation after lysis.

1. Add 4 volumes of buffer **Lyse RBC** to a fresh blood. Mix by inverting the tube.

**Note 1:** For example, if the starting blood volume is 300 µl, add 1200 µl of Lyse RBC buffer.

**Note 2:** The maximum amount of blood is 1.5 ml.

**Note 3:** Do not use frozen blood.

**Note 4:** Use a diluted buffer Lyse RBC.

2. Keep at 4°C for 10 minutes to lyse erythrocytes. Mix twice by inverting the tube.

3. Centrifuge at 400 x g for 10 minutes at 4°C, and carefully decant the supernatant.

**Note 1:** Carefully pipette to collect the rest of the supernatant.

4. Add two volumes of **Lyse RBC** to the leukocytes pellet. Mix thoroughly by vigorous vortexing.

**Note 1:** For example, if the starting blood volume is 300 µl, add 600 µl of Lyse RBC buffer.

5. Centrifuge at 400 x g for 10 minutes at 4°C, and carefully decant the supernatant.

**Note 1:** Carefully pipette to collect the rest of the supernatant.

6. Add 500 µl buffer **RL** to the leukocytes pellet. Mix thoroughly by pipetting for homogenization.

**Note 1:** Ensure that β-ME is added to buffer RL.

7. Carefully transfer the sample to the homogenization spin-column placed in a 2 ml receiver tube. Centrifuge at maximum speed for 2 minutes.

**Note 1:** Centrifugation through the homogenization spin-column filtrates and homogenizes the lysate and removes DNA.

8. Add 300 µl 96-100 **%** ethanol to the flow-through**.** Mix thoroughly by pipetting. Do not centrifuge.

**Note 1:** A precipitate may form after addition of ethanol.

9. Apply the sample, including any precipitate, to the RNA binding spin-column placed in a 2 ml receiver tube. Centrifuge for 1 min at 11000 x g. Discard the flow-through.

10. Add 500 µl **Wash RB1** buffer and centrifuge at 11000 x g for 1 minute.

11. Remove the spin-column, pour off supernatant and place back into the receiver tube.

12. Add 600 µl **Wash RBW** buffer and centrifuge at 11000 x g for 1 minute.

13. Remove the spin-column, pour off supernatant and place back into the receiver tube.

14. Add 350 µl **Wash RBW** buffer and spin down at 11000 x g for 2 minutes.

**Note 1:** Be careful not to contaminate the sample while removing spin-column from receiver tube. Check whether membrane is completely dry. If not, pour off supernatant and place back spin-column into the receiver tube and spin down for additional 1 minute.

15. Place spin-column into new receiver tube (1.5-2 ml) and add 40-60 µl RNase-free water directly onto the membrane.

**Note 1:** It is not necessary to close the tube at this step..

16. Centrifuge for 1 min at 11000 x g.

17. Remove spin-column, cap the receiver tube. RNA is ready for analysis/manipulations. It can be stored either at 2÷8°C (preferred) or at -20°C (avoid multiple freezing and defrosting of RNA).

**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 Human Blood RNA Purification Kit** is designed for rapid purification of total RNA from fresh human blood. Purified RNA is free of contaminants, such as: DNA, proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others. In the first step the red blood cells are lysed and the remaining leukocytes are spun. Then leukocytes are lysed in the presence of denaturing buffer, which inactivates cellular RNases. In the next stage, homogenization spin-columns shear genomic DNA, reducing viscosity of the lysate and eliminating DNA fragments. Then sample is applied to a binding spin-column where all RNA molecules are adsorbed to the matrix and contaminants are efficiently washed away. High-quality RNA is then eluted in RNase-free water. With a good performance we get a total RNA longer than 200 bases. However, it is possible to purify RNA molecules smaller than 200 bases, with gradually decreasing efficiency. Isolated RNA is ready for downstream applications without the need for ethanol precipitation.