EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Human Blood RNA Purification Kit

Kit for isolation of total RNA from fresh human blood

Cat. no. E3596

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Introductory Notes

NOTE 1 • This kit is designed for isolation of total RNA (longer than 200 bases) from fresh human blood. **The kit is not suitable for isolation of RNA from frozen blood.** For RNA isolation from stored or frozen blood samples, use the Universal Blood RNA Purification Kit (Cat. no. E3594). In this case the blood must be stabilized with additional buffer included in the kit.

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 the gene transcription. 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, Citrate- or EDTA- stabilized blood samples.

NOTE 3 • Maximum sample volume is 1.5 ml of blood.

NOTE 4 • The total RNA binding capacity is 125 µg per spin-column. The maximum volume of the column reservoir is 650 µl. Avoid overloading the mini columns. Overloading will significantly reduce yield and purity and may block the mini columns.

NOTE 5 • After freezing, the blood is not suitable for isolation of RNA using the kit.

NOTE 6 • **Lyse RBC** buffer is supplied as a 5x concentrate. Prior to use, pour the content of RNase-free water 100 ml into Lyse RBC 5x bottle. Mark that Lyse RBC is ready to use (Lyse RBC 1x). See label on the buffer.

NOTE 7 • Contaminating RNases are inactivated by addition of reducing agents capable of disrupting disulfide bonds, such as ß-mercaptoethanol (ß-ME) or dithiothreitol (DTT). To promote reduction of disulfide bonds, add 10 µl ß-ME per 1 ml of buffer RL before use. Upon addition of ß ME, RL buffer remains stable for 1 month. A less toxic but more expensive alternative to ß-ME is, to add 10 µl of [1 M] DTT in RNase-free water per 1 ml buffer RL before use. DTT is not stable in buffer RL, thus DTT-supplemented RL buffer aliquots must not be stored. Working aliquots of [1 M] DTT stock solution in RNase-free water must be stored at -20°C for maintaining stability. To set up a [1 M] DTT stock solution (MW = 154.25 g mol^{-1}), dissolve 1.54 g DTT per 10 ml RNase-free water and store in aliquots for one-time usage.

NOTE 8 • All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. Store the components of the kit at 15–25°C with the exception of Lyse RBC. Lyse RBC buffer should be kept at 2–8°C.

NOTE 9 • To obtain RNA of the highest purity it is important to follow carefully the protocol provided below. During the procedure, work quickly. All steps should be performed at room temperature.

Content	25 preps E3596-01	Storage/Stability
Buffer A	0.9 ml	15-25°C
RL	15 ml	15-25°C
Lyse RBC 5x *	2 x 25 ml	2-8°C
RNase-free water	2 x 100 ml	15-25°C
Wash RB1	15 ml	15-25°C
Wash RBW	27 ml	15-25°C
RNase-free water	3 ml	15-25°C
Homogenization Columns	25	15-25°C
RNA Binding Columns	25	15-25°C
Protocol	1	

* Lyse RBC buffer is supplied as a 5x concentrate. Prior to use, add the appropriate amount of RNase-free water (see label on the buffer: to 25 ml Lyse RBC 5x add 100 ml RNase-free water).

Equipment and reagents to be supplied by user

1. β-mercaptoethanol (14.3 M, β-ME) or [1 M] Dithiothreitol (DTT) in RNase-free water, 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.

Protocol

Sample Disruption, Lysis, Homogenization and RNA Binding

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

   - *Addition of Buffer A 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 4 volumes of buffer **Lyse RBC** to a fresh blood. Mix by inverting the tube.

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

   - *The maximum amount of blood is 1.5 ml.*

   - *Do not use frozen blood.*

   - *Ensure proper dilution of buffer Lyse RBC (supplied as 5x concentrate).*

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

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

   - *Carefully pipette to collect the rest of the supernatant.*

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

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

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

   - *Carefully pipette to collect the rest of the supernatant.*

7. Add 400 µl buffer **RL** to the leukocytes pellet. Mix thoroughly by pipetting for homogenization.

   - *Ensure that either β-ME or DTT is added to buffer RL (see page 3, note 7).*

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

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

9. Add 250 µl 96–100% ethanol to the flow-through. Mix thoroughly by pipetting. Do not centrifuge.

   - *A precipitate may form after addition of ethanol.*

10. Apply the sample, including any precipitate, to the **RNA binding spin-column** placed in a 2 ml receiver tube. Centrifuge for 1 min at 11 000 x g. Discard the flow-through.

Washing

11. Add 500 µl **Wash RB1** buffer and centrifuge at 11 000 x g for 1 min.
12. Remove the spin-column, pour off supernatant and place back into the receiver tube.
13. Add 600 µl **Wash RBW** buffer and centrifuge at 11 000 x g for 1 min.
14. Remove the spin-column, pour off supernatant and place back into the receiver tube.
15. Add 300 µl **Wash RBW** buffer and spin down at 11 000 x g for 2 min.

    - *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 min.*

Elution

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

    - *It is not necessary to close the tube at this step.*

17. Centrifuge for 1 min at 11 000 x g.
18. 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).

Quality Control of Isolated RNA

Quality of isolated total RNA is routinely assessed

— **for purity:** By spectrophotometric measurement

Criteria for high quality RNA:

- A260/A280 ratio 1.8–2.1 (high quality range)
- RNA concentration: A260 = 1 ≈ 40 ng / µl x sample dilution factor

— **for physical integrity:** By agarose gel electrophoresis (1–1.5% [w/v]) — quick check.

Criteria for high quality RNA:

- Distinctly visible, prominent and sharp 28S and 18S rRNA bands, no visible band retardation (indicative for RNase action),
- 28S/18S rRNA band intensity ratio is ≥ 2:1,
- the "smear" appearing among the prominent 28S and 18S rRNA bands represents mRNAs of various sizes and spans the region between both prominently visible bands (the "smear" should not concentrate in the small-sized-RNA region),
- no enrichment of small or very small RNAs is observed (<100 nt), which would be indicative for RNA degradation,
- no visible band appears at ~20 kb (indicative for contamination by genomic DNA),
- total RNA appears in distinct, but tissue-specific patterns.

NOTE 1 · ssRNA Migration Behaviour. 28S rRNA and 18S rRNA are single-stranded molecules, and, due to their biological function, form extensive, stable secondary structures. Both rRNAs will therefore migrate faster through a non-denaturing agarose gel, as compared to a dsDNA molecular weight standard. A direct size comparison of rRNAs to dsDNA marker is thus not possible. However, both ribosomal RNA bands are easily and unambiguously identified due to their prominent intensity and appearance. In contrast, precise RNA size comparisons would require much more work-intense denaturing polyacrylamide gel electrophoresis. For the sole application within a mere routine quality control, the extra workload for preparing and running PAGE gel electrophoresis is, in our eyes, not justified. PAGE gel electrophoresis would add no surplus of relevant information in this application.

NOTE 2 · 28S/18S rRNA Band Intensity Ratio > 2:1. In living cells, 28S rRNA and 18S rRNA, respectively, are always present in a 1:1 stochiometric ratio. Since 28S rRNA is more than double the size of 18S rRNA (for Homo sapiens: 28S rRNA ~5000 nt, 18S rRNA ~1900 nt; similar values apply throughout the animal kingdom): The gel band representing 28S rRNA is supposed to appear with more than double of the intensity as compared to its 18S rRNA counterpart. Thus, a 28S / 18S rRNA ratio of <2 and / or visible band retardation are indicative for RNA degradation.

Safety Information

Buffer A

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.

Wash RB1

Warning

H226 Flammable liquid and vapour.
H302 Harmful if swallowed.
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.
P301+**P312** If swallowed: call a poison center/ doctor if you feel unwell.
P403+**P235** Store in a well-ventilated place. Keep cool.
EUH032 Contact with acids liberates very toxic gas.

RL

Warning

H302+**H332** Harmful if swallowed or if inhaled.
H412 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
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.
EUH032 Contact with acids liberates very toxic gas.

Wash RBW

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.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

			ISOLATION OF DNA																		
			E3600	E3585	E3540	E3580	E3510	E3545	E3560	E3555	E3525	E3520	E3595	E3535	E3500	E3565	E3515	E3570	E3575	E3530	E3550	E3551
			MICELLULA DNA[2]	GRAM PLUS & YEAST GENOMIC DNA	AGAROSE – OUT DNA	BACTERIAL & YEAST GENOMIC DNA	BIO – TRACE DNA	BASIC DNA	BONE DNA	CELL CULTURE DNA	FOOD EXTRACT DNA	PCR / DNA CLEAN-UP	PLANT & FUNGI DNA	AGROBACTERIUM PLASMID DNA	PLASMID MINIPREP DNA	QUICK BLOOD DNA	SHORT DNA CLEAN-UP	SOIL DNA	STOOL DNA	SWAB-EXTRACT DNA	TISSUE DNA	TISSUE & BACTERIAL DNA
			AVAILABLE NUMBER OF ISOLATION (PREPS)																			
			50 150	25 100	50 150	50 150	25 100	50 150	25 50	50 150	25 100	50 150	50 150	50 150	50 150	50 150	25 100	50 100	50 100	25 100	50 150	50 150
DNA	GENOMIC	BACTERIA		●		●																●
		YEAST		●		●																
		CELL CULTURE								●											●	●
		PLANT											●									
		FUNGI											●									
		PLANT RICH IN POLYSACCHARIDES [1]											●									
		BLOOD														●						
		SOIL																●				
		STOOL																	●			
		SWAB																		●		
		ANIMAL TISSUES																			●	●
		FFPE TISSUE SECTIONS																			●	●
		RODENT TAILS																			●	●
		HAIR																			●	●
		INSECTS																			●	●
		URINE																			●	●
		BONE							●													
		BIOLOGICAL TRACES					●															
		FOOD									●											
	PLASMID	BACTERIA						●						●	●							
		YEAST				●																
	ISOLATION FROM AGAROSE GELS				●			●														
	PURIFICATION OF PCR PRODUCTS / DNA AFTER ENZYMATIC REACTIONS		●					●				●					●					

All kits contain buffers WASH in ready to use form

1. Additionally required lyse CT buffer (E0324)
2. Kit for creation of emulsions and subsequent DNA purification.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

				ISOLATION OF RNA					
				E3700	E3594	E3596	E3598	E3599	E3593
				RNA EXTRACOL [2]	UNIVERSAL BLOOD RNA	HUMAN BLOOD RNA	UNIVERSAL RNA	UNIVERSAL RNA /miRNA	FFPE RNA Purification Kit
				PREPS					
				25 100	25	25	25 100	25 100	25 100
RNA	TOTAL RNA LONGER THAN 200 BASES	ANIMAL TISSUE					●	●	
		PLANT TISSUE					●	●	
		BACTERIA					●		
		YEAST					●		
		CELL CULTURE					●	●	
		HUMAN BLOOD	FRESH	●	●	●	●		
			FROZEN [1]		●				
		ANIMAL BLOOD	FRESH	●	●				
			FROZEN [1]		●				
	miRNA OR TOTAL RNA	ANIMAL TISSUE		●				●	
		FFPE TISSUE SECTIONS							●
		PLANT TISSUE		●				●	
		CELL CULTURE		●				●	
		BACTERIA		●					
		YEAST		●					
		BLOOD/LEUKOCYTES		●					
	PURIFICATION OF RNA AFTER ENZYMATIC REACTIONS						●	●	
	ON-COLUMN DNase DIGESTION				●		●		

All kits contain buffers WASH in ready to use form

1. Frozen with the addition of Lyse Blood buffer (included in kit).
2. Phenol-based reagent for isolation 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.

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