EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Viral RNA/DNA Purification Kit

Kit for simultaneous purification of viral RNA / DNA from plasma, serum and cell-free body fluids.

Cat. no. E3592

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Table of Contents

Equipment and reagents to be supplied by user:

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5-2 ml tubes, a heating block capable of incubation at 60°C, vortex.
- Ethyl alcohol [96-100% v/v]. For samples <200 µl: 0.9% NaCl solution.

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for simultaneous isolation of viral RNA and DNA longer than 200 nt. Isolation of shorter fragments is possible, however, they are isolated with reduced efficiency. The kit allows isolation of RNA / DNA from plasma, serum or other cell-free body fluids.

NOTE 2 · Maximum Sample Amount. The procedure is optimized for use with a starting volume of 200 µl. Whenever a smaller volume sample is used, bring the volume up to 200 µl with a 0.9% NaCl solution (free of RNases / DNases).

NOTE 3 · Sample Storage. After collection, plasma or serum can be stored for up to 5 hours at 2-8°C. For longer storage it is recommended to freeze samples at -20°C or -80°C in aliquots. Frozen plasma or serum samples must not be thawed more than once.

NOTE 4 · Carrier RNA. The addition of carrier RNA enhances binding of viral nucleic acids to membranes. This is particularly important in cases where the sample contains very few target molecules. In addition, the introduction of a large amount of carrier RNA reduces the chance of viral nucleic acids degradation. Add 150 µl RNase free water to the tube containing 150 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Do not freeze–thaw the aliquots of carrier RNA more than 3 times. [Carrier RNA: Poly(A) 2000-10000 nt.]

NOTE 5 · Yield of viral nucleic acids. The amount of viral nucleic acids isolated from biological samples is usually below 1 µg and is not suitable for spectrophotometric measurement. Quantitative amplification methods are recommended for determination of yields. When quantifying isolated nucleic acids remember that there will be considerably more carrier RNA in the sample than viral RNA.

NOTE 6 · Internal controls. Commercially available amplification systems may require the introduction of an internal control into the purification procedure. Internal control RNA or DNA should be added together with the carrier RNA to the lysis buffer. For optimal purification efficiency, internal control molecules should be longer than 200 nucleotides.

NOTE 7 · Additional recommendations. In order to avoid cross-contamination of samples during isolation and centrifugation, particular care should be taken not to wet the rim of the column during the application of the lysate and wash buffers.

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

NOTE 9 · 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, follow carefully the protocol provided below.

Protocol

Part I Before starting

Dissolve Carrier RNA. Add 150 µl RNase free water to the tube containing 150 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl. Dissolve the carrier RNA thoroughly, divide it into conveniently sized aliquots, and store it at –20°C. Use 5 µl (5 µg) of carrier RNA per one prep.

All centrifugation steps should be carried out at room temperature.

1. Apply 25 µl of activation **Buffer A** onto the **binding 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 A onto the center of the resin enables complete wetting of membranes and maximal binding of DNA/RNA.*

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

2. Equilibrate samples to room temperature.

3. Add 5 µl carrier RNA suspended in RNase free water to 215 µl Sol V buffer.

   - *Carrier RNA does not dissolve in Sol V buffer. It must be dissolved in RNase free water first and then added to Sol V buffer.*

   - *The given proportion refers to one isolation. A larger volume of the mixture can be prepared according to the amount of isolation carried out.*

Part II RNA/DNA isolation

1. Pipet 20 µl **Proteinase K** into a 1.5-2 ml Eppendorf tube.

2. Add 200 µl of plasma or serum into the tube with **Proteinase K**.

   - *If the sample volume is less than 200 µl, add the appropriate volume of 0.9% sodium chloride solution.*

3. Add 220 µl Sol V buffer (containing carrier RNA). Close the cap and mix thoroughly by inverting the tube or vortex.

   - *Do not add Proteinase K directly to Sol V buffer.*

4. Incubate at 60°C for 15 min in a heating block.

   - *During the incubation period, mix by occasionally inverting the tube several times.*

5. To remove drops of solution from the lid, centrifuge the tubes briefly at low speed.

6. Add 250 µl of ethanol (96-100%) and mix thoroughly. Incubate the lysate with the ethanol for 1 min at room temperature.

7. Carefully transfer the sample to the **binding spin-column** placed in a 2 ml receiver tube. Close the cap and centrifuge at 8 000 x g for 1 min. Place the spin-column into the clean receiver tube and discard receiver tube containing filtrate.

8. Carefully open the column, add 500 µl Wash V1 buffer. Close the cap and centrifuge at 8 000 x g for 1 min. Place the spin-column into the clean receiver tube and discard receiver tube containing filtrate.

9. Carefully open the column, add 500 µl **Wash RBW** buffer. Close the cap and centrifuge at 8 000 x g for 1 min.

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

    - *Be careful not to contaminate the sample while removing the spin-column from the receiver tube. Check, whether the membrane of the spin column is completely dry. If not, pour off any remaining supernatant and place back spin-column into the receiver tube. Spin down for one additional min.*

11. Centrifuge at full speed for 2 min to dry the membrane completely.

12. Place spin-column in a clean microcentrifuge tube (1.5-2 ml) and add 50-100 µl **RNase-free water** directly onto the membrane.

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

13. Incubate spin-column for 2 min at room temperature and centrifuge at full speed for 2 min.

14. Remove spin-column, cap the receiver tube. RNA/DNA is ready for analysis/manipulations. Isolated RNA/DNA can be stored either at 2-8°C (preferred) or at -20°C. Avoid multiple freezing and thawing.

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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 Viral RNA/DNA Purification Kit is designed for simultaneous isolation of viral RNA and DNA. The procedure is suitable for use with plasma, serum, and other cell-free body fluids. Samples can be either fresh or frozen, assuming that they have not been frozen and thawed more than once.***

The procedure is optimized for use with a starting volume of 200 µl. A simple protocol based on four steps (lysis, binding, washing and elution) minimizes the time of isolation and the possibility of contamination of samples. The addition of carrier RNA supports the binding of even small amounts of viral nucleic acids. During short centrifugation RNA / DNA binds to the membrane. Unbound impurities remain in the column flow-through. Traces of contaminants remaining on the membrane are efficiently removed in two wash steps. The elution of purified RNA / DNA is carried out with RNase-free water. Effectively purified are nucleic acids with a length of over 200 nt. Isolation of shorter fragments is possible, however, they are isolated with reduced efficiency. Purified nucleic acids are free of proteins, nucleases, and other impurities and are ready for use in amplification reactions or storage at -20°C.

- **GeneMATRIX is a synthetic, new generation DNA- and RNA-binding membrane, selectively binding nucleic acids to composite silica structures. Novel binding and washing buffers were developed to take full advantage of GeneMATRIX capacity, yielding biologically active, high-quality nucleic acids.**

The matrix is conveniently pre-packed in ready-to-use spin-format. Due to the unique chemical composition of the matrices, in combination with optimized spin-column design, nucleic acids are isolated in outstanding quality and high purity. To speed up and simplify the isolation procedure, the key buffers are colour coded, allowing for monitoring complete mixing of mission-critical solutions, thus aiding to render the purification procedure even more reproducible.

As a result, we offer kits, containing matrixes and buffers that guarantee rapid, convenient, safe and efficient isolation of ultrapure nucleic acids. Isolated 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. One additional advantage is the high level of matrix performance reproducibility, as all components are prepared inhouse at Eurx Ltd.

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