EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Tick RNA/DNA Purification Kit

Kit for simultaneous purification of RNA/ DNA from ticks

cat. no. E3590

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Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for simultaneous isolation of viral and bacterial RNA and DNA. The use of Tissue Grinding Tool with sharp grinding beads allows for isolation of RNA / DNA from arachnids such as ticks and other insects.

NOTE 2 · Maximum Sample Amount. Nucleic acids from one tick/insect used in one miniprep. The insect is grinded and lysed by Sol V.

NOTE 3 · Sample Storage. After collection, ticks/insects can be stored for up to 48 hours at 2-8°C. For longer storage it is recommended to freeze samples at -20°C or -80°C. 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 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 Sol V buffer.

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 RNA/DNA, follow carefully the protocol provided below.

Content	25 preps E3590-01	Storage/Stability
Buffer A	0.75 ml	15-25°C
Sol V	6.5 ml	15-25°C
Wash V1	15 ml	15-25°C
Wash RBW	15 ml	15-25°C
Proteinase K (20 mg/ml)	0.6 ml	–20°C
RNase-free water	4.5 ml	15-25°C
Carrier RNA	150 µg*	15-25°C
DNA/RNA Binding Columns	25 szt.	15-25°C
Filtration Spin Columns	25 szt.	15-25°C
Tissue Grinding Tool	25 szt.	15-25°C
Protocol	1	

* Add 150 µl RNase free water to the tube containing 150 µg lyophilized carrier RNA to obtain a solution of 1 µg/µl.

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], 0,9 % NaCl or 1x PBS (no magnesium, no calcium).

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 transferring 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. Spin down shortly the garnet particles to the bottom of the tube.

4. Add 5 µl **Carrier RNA** suspended in RNase free water to 215 µl **Sol V** buffer.

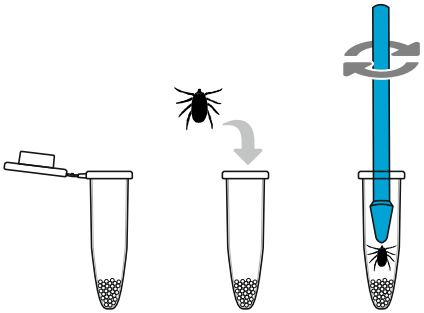
   - *Carrier RNA does not dissolve in Sol V buffer. It must be dissolved in RNase free water first and then added to Sol V buffer.*

   - *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. Add 220 µl **Sol V** (containing carrier RNA) to the tube with garnet grinding beads. Then add an arachnid/insect and crush by rotating the pestle with your fingers.

   - *For very small arachnid/insect (e.g. Ixodes ricinus) it is recommended to grind in a smaller volume of Sol V, e.g. 50 µl. In this case, before the addition of Proteinase K, the Sol V volume should be adjusted to 220 µl.*



2. Remove the pestle, add 200 µl of 0,9 % NaCl or 1x PBS and 20 µl of Proteinase K. Vortex for a few seconds or mix thoroughly by inverting the tube.
3. Incubate at 60°C for 30 min.
4. Transfer the mixture together with the garnet grinding beads to the **filtration spin-column** placed in a collection tube to recover the entire lysate.

   - *Pour contents to the filtration spin-column. During centrifugation the lysate will be filtered and further homogenized.*

5. Centrifuge at 10 000 x g for 1 min.
6. Remove **filtration spin-column**. Mix thoroughly by pipetting to resuspend any precipitate that may have formed during the spin down cycle. Add 500 µl of ethanol (96-100%) and mix thoroughly.
7. Carefully transfer 600 µl of 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. Transfer the remaining mixture to the same **DNA binding spin-column** 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 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.

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

11. 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.*

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

13. 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.*

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

15. 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.

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.

Sol V

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.
P337+**P313** If eye irritation persists: get medical advice/ attention.
P333+**P313** If skin irritation or rash occurs: get medical advice/attention.

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.

Wash V1

Warning

H226 Flammable liquid and vapour.
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.
P302+**P352** If on skin: wash with plenty of water.
P332+**P313** If skin irritation occurs: get medical advice/attention.
P305+**P351**+**P338** If in eyes: rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P337+**P313** If eye irritation persists: get medical advice/ attention.
P403+**P235** Store in a well-ventilated place. Keep cool.

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 Tick RNA/DNA Purification Kit is designed for simultaneous isolation of viral and bacterial RNA and DNA that live in small arachnids or insects.

The kit is optimized for the isolation of RNA and DNA from microorganisms (bacteria, viruses, fungi) that live in small (up to 5 mm long) arachnids or insects. A simple protocol enables a fast isolation. The first step is the efficient fragmentation of the arachnid/insect with the use of Tissue Grinding Tool. Then the material is lysed and filtrated. The addition of carrier RNA supports the binding of even small amounts of viral and bacterial 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. Purified nucleic acids have a length of over 25 nt (miRNA). 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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