EURx® MOLECULAR BIOLOGY PRODUCTS

Version 1.0
October 2016

GeneMATRIX Bone DNA Purification Kit

Kit for isolation of DNA from animal or human bones

Cat. no. E3560

EURx Ltd. 80-297 Gdansk Poland
ul. Przyrodnikow 3, NIP 957-07-05-191
KRS 0000202039, www.eurx.com.pl
orders: email: orders@eurx.com.pl
tel. +48 58 524 06 97, fax +48 58 341 74 23

Table of Contents

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the isolation of DNA from animal or human bones and teeth. The isolated DNA can be used as a template in amplification reactions for both genomic and mitochondrial sequences.

NOTE 2 · Maximum Sample Amount. One minicolumn enables purification of DNA from up to 0.4 g of bone sample. The maximum volume of the column reservoir is 650 µl. The maximum column binding capacity for DNA is 25 µg.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature with the exception of Sol BN buffer and Proteinase K. Sol BN buffer should be kept at 2–8°C and Proteinase K at -20°C.

NOTE 4 · 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, stick carefully to the protocol provided below.

Equipment and reagents to be supplied by the experimenter.

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, ethanol 96–100%, a heating block capable of incubation at 56°C.
- Equipment for bones disruption and grinding, depending on the method chosen: mortar and pestle and liquid nitrogen or milling/drilling machine with single-use grinding discs or others metal blenders.

Protocol

1. Remove dirt and if possible the outer surface from the bone sample.

   - *This step removes possible contaminations that can interfere with downstream applications.*

   - *To remove the outer surface use if possible a milling/drilling machine with single-use grinding discs.*

2. Crush the bone into small fragments. Grind fragments under liquid nitrogen to a fine powder using a mortar and pestle or a specialized freezer mill.

   - *Try to obtain as fine a powder as possible. The finer powder, the greater yield of DNA released during the isolation procedure.*

3. Place up to 0.4 g bone sample in 2 ml screw cap tube (provided with the kit).

4. Add 800 µl **Lyse BN** buffer. Suspend the sample thoroughly.

5. Add 40 µl **Proteinase K**. Mix by inverting or vortexing the tube.

6. Incubate with gentle agitation overnight at 56°C.

   - *Lysis time will vary depending on the size and density of the source material. The lysis conditions given here are intended to serve as guidelines.*

7. Apply 30 µl of activation **Buffer BN** onto the 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 BN 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.*

8. Add 800 µl **Sol BN** buffer. Mix thoroughly by inverting or vortexing the tube. Incubate for 10 min at 56°C.

9. Centrifuge the lysate in a microcentrifuge for 3 min at 12 000 x g.

10. Transfer 1200 µl of the supernatant to a new 2 ml microcentrifuge tube and add 600 µl of ethanol (96–100%). Mix thoroughly by inverting the tube or by pipetting.

11. Transfer 600 µl of the lysate to the **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

12. Repeat step 11.

13. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

    - *Continue centrifugation, if not all of the lysate passed through the column.*

14. Add 500 µl of **Wash BNX1** buffer and spin down at 11 000 x g for 1 min.

15. Remove spin-column, pour off supernatant, replace back spin-column.

16. Add 500 µl of **Wash BNX2** buffer and spin down at 11 000 x g for 1 min.

17. Remove spin-column, pour off supernatant, replace spin-column.

18. Spin down at 11 000 x g for 1 min to remove traces of the **Wash BNX2** buffer.

19. Place the spin-column in a new collection tube (1.5–2 ml) and add 30–100 µl of **Elution** buffer preheated to 70°C to elute the bound DNA.

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

    - *The following eluting solutions can be used:*

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

20. Incubate spin-column/receiver tube assembly for 2 min at room temperature.

21. Spin down at 11 000 x g for 1 min.

22. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/ manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

EURx® MOLECULAR BIOLOGY PRODUCTS

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.

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 Bone DNA Purification Kit is designed for rapid purification of DNA (genomic, mitochondrial) from animal or human bones and teeth. Purified DNA is free of contaminants, such as: proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.

A bone sample is finely grinded and the obtained powder is subsequently solubilized by lysis in the presence of special desintegrating buffer, which preserves and stimulates quantitative recovery of all traces of DNA. Further, Proteinase K digests collagen and other proteins. Optimized buffer and ethanol are added to provide selective conditions for DNA binding during brief centrifugation, while contaminants pass through the GeneMATRIX resin in the spin-column. Traces of contaminants remaining on the resin are efficiently removed in two wash steps. High-quality 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.

EURx®
MOLECULAR
BIOLOGY
PRODUCTS

EURx Ltd. 80-297 Gdansk Poland
ul. Przyrodnikow 3, NIP 957-07-05-191
KRS 0000202039, www.eurx.com.pl
orders: email: orders@eurx.com.pl
tel. +48 58 524 06 97, fax +48 58 341 74 23