EURx Molecular Biology Products

GeneMATRIX Bone DNA Purification Kit

Kit for isolation of DNA from animal or human bones

Cat. no. E3560

Version 1.2

March, 2010

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

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

Note 1: 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: One minicolumn enables purification of DNA from up to 0.4 g of bone sample.
Note 3: The kit should be stored at room temperature, with the exception of Sol BN buffer and Proteinase K, Sol BN buffer should be kept at 2÷8ºC and Proteinase K at -20ºC.
Note 4: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.
Note 5: The kit does not contain 96 % ethanol, the reagent needed during the isolation procedure.

Protocol

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

   Note 1: This step removes possible contaminations that can interfere with downstream applications.
   Note 2: To remove the outer surface use if possible a milling/drilling machine with single-use grinding discs.

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

   Note 1: 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 56ºC.

7. Apply 40 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.

   Note 1: Addition of Buffer BN onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.
   Note 2: The membrane activation should be done after overnight incubation, before Sol BN buffer addition.

8. Add 800 µl **Sol BN** buffer. Mix thoroughly by inverting or vortexing the tube.

9. Incubate for 10 min at 56ºC.

10. Centrifuge the lysate in a microcentrifuge for 3 min at 14 000 rpm.

11. Transfer 1200 µl of the supernatant to a new 2 ml microcentrifuge tube.

12. Add 600 µl of **96 % ethanol**. Mix thoroughly by inverting or vortexing the tube.

13. Centrifuge briefly to remove drops from the inside of the tube lid.

14. Transfer 600 µl of the lysate to the spin-column placed in a collection tube.

15. Centrifuge for 30 seconds at 12000 rpm.

16. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

17. Repeat 14-16 steps.

18. Transfer the remaining supernatant to the spin-column placed in a collection tube. Centrifuge for 1 min at 12000 rpm to filtrate the remains of the lysate through the resin.

Note 1: Continue centrifugation, if not all of the lysate passed through the column.

19. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

20. Add 500 µl **Wash BNX1** buffer to the spin-column and centrifuge for 1 min at 12000 rpm.

21. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

22. Add 500 µl **Wash BNX2** buffer to the spin-column and centrifuge for 2 min at 12000 rpm.

23. Place the spin-column in a new collection tube (1.5-2 ml) and add 30-100 µl of **Elution** buffer (10 mM Tris-HCl, pH 8.5) preheated to 70°C to elute the bound DNA.

Note 1: 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 micropippete.

Note 2: The following elution solutions can be used:

1. 5-10 mM Tris-HCl buffer, pH 8.0-9.0
2. 0.5-1 x TE buffer, pH 8.0-9.0 (not recommended for DNA sequencing).
3. Other special application buffers can be used, if their pH and salt concentration is similar to that of 5-10 mM Tris-HCl, pH 8.0-9.0.

24. Incubate the spin-column/collection tube assembly for 5 min at room temperature.

25. Centrifuge the spin-column for 30 seconds at 12000 rpm.

26. Discard spin-column, cap the collection tube. DNA is ready for analysis/manipulation. It can be stored either at 2÷8° C or at -20°C.

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.