EURx® MOLECULAR BIOLOGY PRODUCTS

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# GeneMATRIX Bone DNA Purification Kit

Kit for isolation of DNA from animal or human bones

**Cat. no. E3560**

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# 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.

| Content | 25 preps E3560-01 | 50 preps E3560-02 | Storage/Stability |
|---|---|---|---|
| Buffer BN | 0.9 ml | 1.8 ml | 15-25°C |
| Lyse BN | 24 ml | 48 ml | 15-25°C |
| Proteinase K (20 mg/ml) | 1.2 ml | 2.4 ml | –20°C |
| Sol BN | 24 ml | 48 ml | 2-8°C |
| Wash BNX1 | 15 ml | 30 ml | 15-25°C |
| Wash BNX2 | 15 ml | 30 ml | 15-25°C |
| Elution | 3 ml | 6 ml | 15-25°C |
| DNA Binding Columns | 25 | 50 | 15-25°C |
| Screw cap tubes | 25 | 50 | 15-25°C |
| Protocol | 1 | 1 | |

## *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.

# Safety Information

## Lyse BN

**Warning**

**H319** Causes serious eye irritation.
**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.
**P337**+**P313** If eye irritation persists: 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.

## Buffer BN

**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 BN

**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.
**P333**+**P313** If skin irritation or rash occurs: get medical advice/attention.
**P337**+**P313** If eye irritation persists: get medical advice/ attention.
**EUH032** Contact with acids liberates very toxic gas.

## Wash BNX1

**Warning**

**H226** Flammable liquid and vapour.
**H302**+**H332** Harmful if swallowed or if inhaled.
**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.
**P301**+**P312** If swallowed: call a poison center/ doctor if you feel unwell.
**P302**+**P352** If on skin: wash with plenty of water.
**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.

## Wash BNX2

**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.

## *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.

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