EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Bio-Trace DNA Purification Kit

Kit for isolation of DNA from biological traces

Cat. no. E3510

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Table of Contents

Content	25 preps E3510-01	100 preps E3510-02	Storage/Stability
Buffer BT	0.9 ml	3.6 ml	15-25°C
Lyse BT	11 ml	42 ml	15-25°C
Proteinase K (20 mg/ml)	0.6 ml	2.4 ml	–20°C
Sol BT	11 ml	42 ml	2-8°C
Wash BTX1	15 ml	60 ml	15-25°C
Wash BTX2	15 ml	60 ml	15-25°C
Elution	3 ml	12 ml	15-25°C
DNA Binding Columns	25	2 x 50	15-25°C
Filtration Columns	25	2 x 50	15-25°C
Protocol	1	1	

Introductory Notes

NOTE 1 · Kit Specification. The Bio-Trace Kit is designed for isolation of DNA from the traces of biological samples, such as: fresh or frozen blood, blood spot, saliva, semen spots, hair roots and stems, cigarette filters, chewing gum, fragments of tissues, urine, among others. The kit is effective in DNA purification from both fresh samples and dried, many years old or preserved for example in alkohol or formaline.

NOTE 2 · Maximum Sample Amount. The maximum column binding capacity for DNA is 25 µg. The maximum volume of the column reservoir is 650 µl.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of Sol BT buffer and Proteinase K. Sol BT 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. Tissue lysates are very sticky. This can lead to slow lysate filtration through the resin. Therefore it is advisible to check, if lysate and washes passed completely through the resin.

NOTE 5 · Elution buffer is a low salt solution, that contains no metal ion chealators (e.g. EDTA) that can inhibit subsequent enzymatic reactions. Elution buffer composition is suitable for downstream applications such as digestion with restriction enzymes, phosphorylation, ligation, Sanger sequencing, NGS etc. It is also possible to elute the DNA with Tris-HCl, water or TE.

Equipment and reagents to be supplied by the experimenter

- Microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml tubes, a heating block capable of incubation at 37–70°C.
- [1 M] Dithiothreitol (DTT), ethanol [96–100% v/v], xylene and PBS. To prepare sterile PBS, dissolve 8 g NaCl, 0.2 g KCl, 1.44 g Na_2HPO_4 and 0.24 g KH_2PO4 in 800 ml H_2O. Adjust pH to 7.4 with HCl. Add H_2O to 1 liter.

Protocol

I. DNA binding spin-columns activation

1. Apply 30 µl of activation **Buffer BT** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column.

   - *Addition of Buffer BT 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. The minimum activation time is 5-15 min.*

II. Sample preparation

A. 1–100 µl of blood

1. Add to the 2 ml Eppendorf tube: 1–100 µl of fresh or frozen blood, 250 µl **Lyse BT** buffer and 10 µl **Proteinase K**.

   - *If the blood volume is less than 100 µl, fill up with PBS to 100 µl.*

2. Mix by inverting the tube several times or by vortexing.

3. Follow the point 1. Part III. of the DNA isolation protocol.

B. Spots of blood, saliva, semen and other biological liquids

1. Cut off the fragment of spotted material (do not exceed 1 cm^2). Cut the material into small pieces. Place the pieces in the 2 ml Eppendorf tube.

   Spots from solid surfaces should be scratched and poured into the 2 ml Eppendorf tube.

2. Add 350 µl **Lyse BT** buffer and 10 µl **Proteinase K**. In the case of semen add additionally 20 µl 1M DTT.

3. Mix thoroughly by inverting the tube and incubate for 60 min at 56°C. Mix by inverting every 15 min.

4. Follow the point 1. Part III. of the DNA isolation protocol.

C. Hair roots and stems, nails

1. Cut off 0.5–1 cm fragments of hair including the roots or cut hair stems into 0.5–1 cm long pieces (if the sample does not contain hair roots).

   Cut nails into small pieces (less than 2 mm^2).

2. Place cut hair or nails (maximum sample size: 20 mg) in 2 ml Eppendorf tube. Add 350 µl **Lyse BT** buffer, 20 µl 1M DTT and 20 µl **Proteinase K**.

3. Mix by inverting the tube several times or by vortexing, then centrifuge to collect hair or nails at the bottom of the tube. Incubate hair roots or hair stems for at least 60 min at 56°C (untill completely digested). Mix by inverting the tube every 30 min. In the case of nails overnight incubation is recommended.

   - *While isolating DNA from hair sample containing hair roots, make sure the roots are completely submerged in the lysis buffer. If necessary, submerge the parts of hair containing roots in the lysis buffer using a sterile tips.*

4. Follow the point 1. Part III. of the DNA isolation protocol.

D. Cigarette filters, chewing gum

1. Remove outer paper from the filter. Cut the paper into small pieces and place the sample in the 2 ml Eppendorf tube.

   Chewing gum (maximum sample size: 50 mg) cut into small pieces and place in the 2 ml Eppendorf tube.

2. Add 350 µl **Lyse BT** buffer and 10 µl **Proteinase K**.

3. Mix by inverting the tube several times or by vortexing and incubate for 60 min at 56°C. Mix by inverting every 15 min.

4. Follow the point 1. Part III. of the DNA isolation protocol.

E. Fragments of tissues

1. Cut the tissue fragment (maximum sample size: 10 mg) into small pieces and place the sample in the 2 ml Eppendorf tube. Suspend the sample thoroughly in 350 µl **Lyse BT** buffer. Add 20 µl **Proteinase K**.

2. Mix by inverting the tube several times or by vortexing and incubate for 3–6 h at 56°C, mix by inverting every 15 min.

   - *After incubation the tissue suspension should be completely digested and appear as transparent fluid. If not, continue the incubation.*

3. Follow the point 1. Part III. of the DNA isolation protocol.

F. Paraffin-embedded tissues

1. Prepare a small section (up to 25 mg) from block of embedded tissue. Place the sample in 2 ml Eppendorf tube.
2. Add 1 ml xylene. Vortex vigorously. Incubate at room temperature for 15 min.
3. Centrifuge for 3 min at 11 000 x g. Remove supernatant by pipetting.
4. Add 1 ml xylene to the pellet, mix by vortexing.
5. Centrifuge for 3 min at 11 000 x g. Remove supernatant by pipetting.
6. Add 1 ml 96% ethanol to the pellet. Mix by vortexing or inverting the tube.
7. Centrifuge for 3 min at 11 000 x g. Remove supernatant by pipetting.
8. Repeat steps 6–7 once.
9. Incubate the open tube at 37°C until the ethanol has evaporated (app. 15 min).
10. Resuspend the tissue pellet in 350 µl **Lyse BT** buffer. Add 20 µl **Proteinase K**.
11. Follow the point 2. of E. Fragments of tissues protocol.

G. Formalin-fixed tissues

1. Wash tissue sample twice with PBS to remove fixative. Discard PBS.
2. Cut tissue fragment (up to 25 mg) into small pieces. Place the sample in 2 ml Eppendorf tube and add 350 µl **Lyse BT** buffer and 20 µl **Proteinase K**.
3. Follow the point 2. of E. Fragments of tissues protocol.

H. Urine

1. Add 2 ml of urine to the 2 ml Eppendorf tube.
2. Centrifuge urine in microcentrifuge for 2 min at 6000 x g.
3. Carefully discard the supernatant without disturbing the pellet. Add to the pellet 350 µl **Lyse BT** buffer, 20 µl 1M DTT and 10 µl **Proteinase K**.
4. Vortex for 15 sec.
5. Incubate for 60 min at 56°C, mix by inverting the tube every 15 min.
6. Follow the point 1. Part III. of the DNA isolation protocol.

III. DNA isolation

1. Add 350 µl of **Sol BT** buffer and mix thoroughly by several times inverting the tube.
2. Incubate for 10 min at 70°C.
3. In the case of biological material samples mentioned in sections:

   A do not add of ethanol
   B, **D**, **H** add 180 µl of ethanol (96–100%)
   C add 230 µl of ethanol (96–100%)
   E, **F**, **G** add 350 µl of ethanol (96–100%)
4. Mix thoroughly by several times inverting the tube.
5. Centrifuge for 2 min at 12 000 x g.
6. Transfer 600 µl of supernatant to the **DNA binding spin-column**, placed in the collection tube. In the case of isolation of DNA from dried spots (blood, saliva or semen) on fabric-type material, transfer the supernatant to the **DNA binding spin-column**, placed in the collection tube (do not exceed the total volume of 600 µl of the supernatant). The soaked material with the remainings of the supernatant left in the tube after lysis should be transfered to the **filtration spin-column** to recover the entire lysate. Centrifuge **filtration spin-column** for 2 min at 11 000 x g. Save the flow-through for the following isolation procedure.
7. Centrifuge **DNA binding spin-column** for 1 min at 11 000 x g.
8. Remove the spin-column, discard flow-through and place back spin-column in the collection tube.
9. Transfer the remaining supernatant or the remaining flow-through from the **filtration spin-column** to the **DNA binding spin-column**, placed in the collection tube. Repeat centrifugation for 2 min at 11 000 x g to pass completely the lysate through the resin.

   - *Continue centrifugation, if not all of the lysate passed through the column.*
10. Take out **DNA binding spin-column**, discard flow-through and place back spin-column in the collection tube.
11. Add 500 µl of **Wash BTX1** buffer and centrifuge for 1 min at 11 000 x g.
12. Take out **DNA binding spin-column**, discard flow-through and place back spin-column in the collection tube.
13. Add 500 µl of **Wash BTX2** buffer and centrifuge for 1 min at 11 000 x g.
14. Spin down at 11 000 x g for 1 min to remove traces of **Wash BTX2** buffer.

15. Place **DNA binding spin-column** in a new collection tube (1.5–2 ml) and add 50 µl of **Elution** buffer (10 mM Tris-HCl, pH 8.5) heated to 70°C to elute 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.*

16. Incubate **DNA binding spin-column**/collection tube assembly for 5 min at room temperature.

17. Centrifuge for 1 min at 11 000 x g.

18. Remove 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.

Safety Information

Buffer BT

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.

Lyse BT

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.

Sol BT

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 BTX1

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 BTX2

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 Bio-Trace DNA Purification Kit is designed for rapid purification of total DNA (genomic, mitochondrial) from the traces of biological samples (especially forensic case work samples), examplified by: fresh or frozen blood, blood spot, saliva, semen spots, hair roots and stems, cigarette filters, chewing gum, fragments of tissues, urine.

The kit is effective in DNA purification from both fresh samples and dried, many years old or preserved for example in alkohol or formaline. Purified DNA is free of contaminants, such as: proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others. Biological trace sample is solubilized/lysed in the presence of special buffer, which desintegrates remaining tissue- and cellular structures, while preserving integrity and stimulating quantitative recovery of all traces of DNA spontaenously released during progressing decay of older samples. Further, Proteinase K digests contaminating proteins, including stripping-off DNA of all bound proteins, among them nucleases. 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 contamints remaining on the resin are efficiently removed in two wash steps. High-quality cellular 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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