EURx Molecular Biology Products

GeneMATRIX Food-Extract DNA Purification Kit

Kit for isolation of DNA from fresh and processed food
of plant and animal origin

Cat. no. E3525

Version 5.1

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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 Food-Extract DNA Purification Kit is designed for isolation of DNA from raw or processed food of plant, animal or mixed origin.
Note 2: One minicolumn enables purification of DNA from up to 300 mg sample, however in the case of dry, hygroscopic material (flour, corn flakes, dried plants) it is necessary to reduce sample weight below 100 mg.
Note 3: Once the kit is unpacked, store components at room temperature, with the exception of Sol FE buffer, PR buffer and Proteinase K. Sol FE, PR buffers should be kept at 2÷8° C and Proteinase K at -20° C.
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 and RNase A. These reagents need to be provided by the user.

Protocol

1. Apply 40 µl of activation **Buffer FE** 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 FE onto the center of the resin enables complete wetting of membranes and maximal binding of DNA.
Note 2: The membrane activation should be done before starting isolation procedure.

2. Homogenization of sample.

   a. Grind sample under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place up to 300 mg of sample material in 2 ml Eppendorf tube. Add 750 µl **Res FE** buffer. Suspend the sample thoroughly.

   or

   b. Place up to 300 mg of sample material in a suitably sized vessel for homogenizer. Add 750 µl **Res FE** buffer. Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.

Note 1: Homogenization technique strongly depends on the type of sample. In some cases homogenization is not needed and only precise suspension is sufficent (for example: flour, soya or chicken paste, tomato puree, ketchup).
Note 2: The kit enables purification of DNA from up to 300 mg sample, however in the case of dry, hygroscopic material (flour, corn flakes, dried plants) it is necessary to reduce sample weight below 100 mg.
Note 3: In case of liquid samples (soya sauce, soya milk, etc.) add 300 µl of sample to 2 ml Eppendorf tube.
Note 4: If RNA-free DNA is crucial for downstream applications, add 10 µl of RNase A (10 mg/ml).

3. Add 60 µl **Lyse FE** buffer and 10 µl **Proteinase K**.
4. Mix by several-fold inverting the tube and incubate the mixture for 30 min at 65°C (mix twice during incubation by inverting the tube).
5. Centrifuge the lysate in a microcentrifuge for 5 min at 14 000 rpm.
6. Transfer 400 µl of the supernatant to a new 2 ml microcentrifuge tube.

Note 1: In certain cases material strongly absorbs the buffer, causing difficulties in complete transfering of 400 µl supernatant. In such cases reduce the starting weight of sample or transfer as much liquid as possible, then fill-up to 400 µl with Res FE buffer.

7. Add 400 µl PR buffer. Vortex for 5 seconds and incubate on ice for 5 min.

Note 1: PR buffer precipitates non-DNA organic and inorganic material including cell debris, proteins, inhibitors.

8. Centrifuge for 1 min at 14000 rpm.
9. Transfer 600 µl of the supernatant to a new 2 ml microcentrifuge tube.
10. Add 600 µl **Sol FE** buffer.
11. Add 600 µl of **96% ethanol** and mix thoroughly by several times inverting the tube.
12. Centrifuge briefly to remove drops from the inside of the tube lid.
13. Transfer 600 µl of the supernatant to the spin-column placed in the collection tube.
14. Centrifuge for 30 seconds at 12 000 rpm.
15. Take out spin-column, discard flow-through and place back spin-column in the collection tube.
16. Repeat 13-15 steps.
17. Transfer the remaining supernatant to the spin-column placed in the collection tube. Centrifuge for 1 min at 12 000 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.

18. Take out spin-column, discard flow-through and place back spin-column in the collection tube.
19. Add 500 µl **Wash FEX** buffer to the spin-column and centrifuge for 1 min at 12 000 rpm.
20. Take out spin-column, discard flow-through and place back spin-column in the collection tube.
21. Add 500 µl **Wash FEX** buffer to the spin-column and centrifuge for 2 min at 12 000 rpm.
22. Place spin-column in a new collection tube (1.5-2 ml) and add 50-100 µl **Elution** buffer (10 mM Tris-HCl, pH 8.5) previously heated to 70°C to elute bound DNA.

Note 1: Addition of eluting buffer directly onto the center of the resin improves DNA yield. To avoid transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.

Note 2: The following eluting 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, provided that their pH and salt concentration is similar to that of 5-10 mM Tris-HCl, pH 8.0-9.0.

23. Incubate spin-column/collection tube assembly for 5 min at room temperature.
24. Centrifuge the spin-column for 30 seconds at 12 000 rpm. 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 Food-Extract DNA Purification Kit is designed for rapid purification of DNA from raw or processed food of plant, animal or mixed origin and can be used for the identification of DNA from Genetically Modified Organisms (GMO) in the food. Purified DNA is free of contaminants, such as: proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.

Sample is finely grinded and remaining tissue- and cellular structures are subsequently solubilized by lysis in the presence of special desintegrating buffer, which preserves integrity and stimulates quantitative recovery of all traces of DNA. Further, Proteinase K digests contaminating proteins, including stripping-off DNA of all bound proteins, among them nucleases. Specialized solution is added to precipitate enzyme inhibitors that strongly inhibit downstream applications. Optimized buffer and ethanol provide selective conditions for DNA binding to the DNA binding spin-columns. Contaminants 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. Isolated DNA is ready for downstream applications without the need for the ethanol precipitation.