EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Food-Extract DNA Purification Kit

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

Cat. no. E3525

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Table of Contents

Content	25 preps E3525-01	100 preps E3525-02	Storage/Stability
Buffer FE	0.9 ml	3.6 ml	15-25°C
Res FE	23 ml	90 ml	15-25°C
Lyse FE	1.8 ml	7.2 ml	15-25°C
Proteinase K (20 mg/ml)	0.3 ml	1.2 ml	–20°C
PR	12 ml	48 ml	2-8°C
Sol FE	18 ml	72 ml	2-8°C
Wash FEX	30 ml	120 ml	15-25°C
Elution	3 ml	12 ml	15-25°C
DNA Binding Columns	25	2 x 50	15-25°C
Protocol	1	1	

Equipment and reagents to be supplied by the experimenter

- Ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml collection tubes, a heating block capable of incubation at 65°C. Equipment for sample disruption and homogenization, depending on the method chosen: mortar and pestle and liquid nitrogen or handheld rotor-stator homogenizer. Optional RNase A (10 mg/ml, we recommend using EURx RNase A, cat. no. E1350).

Introductory Notes

NOTE 1 · Kit Specification. 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 · Maximum Sample Portion. 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. 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 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 · Maintaining Good Working Practice. All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes. To obtain high quality DNA, stick carefully to the protocol provided below.

Protocol

1. Apply 30 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.

   - *Addition of Buffer FE 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.*

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 750µl **Res FE** buffer. Homogenize using conventional rotor-stator homogenizer until the sample is homogeneous.

   - *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).*

- *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.*

- *In case of liquid samples (cooking oil, soya sauce, soya milk, etc.) add 300 µl of sample to 2 ml Eppendorf tube.*

- *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 maximum speed.
6. Transfer 400 µl of the supernatant to a new 2 ml microcentrifuge tube.

   - *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 sec and incubate on ice for 5 min.

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

8. Centrifuge for 1 min at maximum speed.
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 sec at 11 000 x g.
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 11 000 x g to filtrate the remains of the lysate through the resin.

    - *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 11 000 x g. Take out spin-column, discard flow-through and place back spin-column in the collection tube.

20. Add 500 µl **Wash FEX** buffer to the spin-column and centrifuge for 1 min at 11 000 x g. Remove spin-column, pour off supernatant, replace spin-column.

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

22. Place the spin-column in a new collection tube (1.5–2 ml) and add 50–100 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 transfering traces of DNA between the spin-columns do not touch the spin-column walls with the micropippete.*

    - *The following elution 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, if their pH and salt concentration is similar to that of 5–10 mM Tris-HCl, pH 8.0–9.0.*

23. Incubate the spin-column/collection tube assembly for 2 min at room temperature.

24. Centrifuge the spin-column for 1 min at 11 000 x g.

25. 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.

Safety Information

Buffer FE

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 FE

Danger

H318 Causes serious eye damage.
H412 Harmful to aquatic life with long lasting effects.
P280 Harmful to aquatic life with long lasting effects.
P273 Avoid release to the environment.
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.

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 FE

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 FEX

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 synonymous for a family of synthetic, new generation, nucleic acid binding membranes.

The GeneMATRIX membrane family has gained fame for two striking features: First, for their extraordinary high binding capacity, allowing to isolate nucleic acids with optimal yield. Second, for their remarkably high specificity. Even compounds of pronounced chemical similarity such as DNA, RNA and polysaccharides are easily differentiated amongst each other by the selectivity of these highly optimized matrices. This feature allows to isolate highly pure nucleic acids, that remain to work reliable even after being subjected to extended storage periods (years). Or, directly upon isolation, when used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybridization, etc ...

We take great care to fine-tune all components and variables of the nucleic acid purification system towards each other – a multi-parameter optimization.

All matrices ship conveniently pre-packed in a ready-to-use spin-column format. Spin columns are specifically constructed for precise adjustment of liquid flow-through rate to optimal values. Novel binding and washing buffers are developed to take full advantage of GeneMATRIX unique capacity, resulting in isolation of biologically active, high-quality nucleic acids. And, last not least, a lot of time and efforts went into development of the various GeneMATRIces, thus providing a platform of unique chemical composition.

High and continuous reproducibility of matrix performance is always warranted, since component preparation as well as stringent quality control is entirely performed in-house at EURx Ltd.

Whatever your experience with nucleic acids isolation kits may look like, most likely you will encounter a difference with GeneMATRIX. And, we are so much convinced, you'll love it. Enjoy.

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.

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