EURx® MOLECULAR BIOLOGY PRODUCTS

Version 1.1
May 2024

GeneMAGNET Food DNA Purification Kit

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

Cat. no. E3427

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Table of Contents

Content	96 preps E3427-01	Storage/Stability
Res FE	90 ml	15-25°C
Lyse FE	7.2 ml	15-25°C
Proteinase K (20 mg/ml)	1.2 ml	–20°C
PR	48 ml	2-8°C
Sol FE	72 ml	2-8°C
Wash FEX	180 ml	15-25°C
Wash FEX2	90 ml	15-25°C
Elution	12 ml	15-25°C
Magnetic Beads	1000 µl	2-8°C
Protocol	1	

Equipment and reagents to be supplied by the user

- Magnetic stand E0361 for 16 tubes, E0362 for 24 tubes, E0363 for 96-well plate. To be purchased separately.
- Ethanol [96 -100% v/v], microcentrifuge, disposable gloves, sterile pipette tips, sterile 1.5 -2 ml collection tubes or 96-well plate with minimal well volume 2 ml, 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. Food DNA Purification Kit is designed for isolation of DNA from raw or processed food of plant, animal or mixed origin with silica-covered magnetic beads by manual or automatic method.

NOTE 2 · Maximum Sample Portion. One preparation enables purification of DNA from up to 300 mg sample, however in the case of dry, hygroscopic material (flour, corn flakes, dried plants) perform the method optimization and, if necessary, reduce sample weight below 100 mg.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of Sol FE buffer, PR buffer, Proteinase K and Magnetic Beads. Sol FE, PR buffers and Magnetic Beads 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.

NOTE 6 · 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.

Protocol

1. 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.

   c) Homogenize the sample, weigh out up to 300 mg of sample in 2 ml Eppendorf tube and add 750 µl Res FE buffer.

   - *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).*

2. Add 60 µl **Lyse FE** buffer and 10 µl **Proteinase K**.
3. 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).
4. Centrifuge the lysate in a microcentrifuge for 5 min at maximum speed.
5. Transfer 400 µl of the supernatant to a new 2 ml microcentrifuge tube.

   - *In certain cases the homogenized material strongly absorbs the buffer, causing difficulties in obtaining 400 µl of 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.*

6. 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.*

7. Centrifuge for 1 min at maximum speed.
8. Transfer 600 µl of the supernatant to a new 2 ml microcentrifuge tube or 96-well plate (working volume at least 2 ml). Continue the protocol by manual method or proceed with automatic method.
9. Add 600 µl **Sol FE** buffer.
10. Add 600 µl of 96% ethanol and mix thoroughly by pipetting or inverting the tube.
11. Resuspend **Magnetic Beads** before removing them from the storage tube by vortexing or pipetting. Add 10 µl of resuspended **Magnetic Beads** to the sample and mix by vortexing or pipetting for 1 min.

    - *If needed 10 µl of Magnetic Beads can be added into 600 µl 96% ethanol (step 10), mixed and added together to the sample.*

12. Separate the magnetic beads against the side of the tubes/wells on magnetic stand. Wait until all the beads have been attached to the magnets.
13. Keeping the samples on magnetic stand, remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 800 µl of **Wash FEX** and mix by pipetting or vortexing resuspending the beads in the solution.
14. Separate the magnetic beads against the side of the tubes/wells on magnetic stand. Wait until all the beads have been attached to the magnets.

15. Keeping the samples on magnetic stand, remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 800 µl of **Wash FEX** and mix by pipetting or vortexing for 10 s.

16. Separate the magnetic beads against the side of the tubes/wells on magnetic stand. Wait until all the beads have been attached to the magnets.

17. Keeping the samples on magnetic stand, remove and discard the supernatant by pipetting.

18. Remove the magnetic stand/transfer tubes to the laboratory rack, add 800 µl of **Wash FEX2** and mix by pipetting or vortexing for 10 s.

19. Separate the magnetic beads against the side of the tubes/wells on magnetic stand. Wait until all the beads have been attached to the magnets.

20. Keeping the samples on magnetic stand, remove and discard the supernatant by pipetting without disturbing magnetic beads. Leave the open tubes/plate in magnetic stand and air dry the beads for 15-20 min.

    - *Wash FEX2 contains alcohol, make sure all the solution evaporates before proceding to the next step.*

21. Add 100 µl **Elution** to the tube/well and mix by pipeting or vortexing for 2 min.

22. Separate the magnetic beads against the side of the wells. After all the beads have been attached to the magnets transfer the supernatant containing the purified DNA to a suitable tube/plate. DNA is ready for analysis/manipulations. Isolated DNA can be stored either at 2-8°C (preferred) or at -20°C.

Safety Information

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 / Wash FEX2

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.

- ***GeneMAGNET Food DNA Purification Kit is designed for rapid purification of DNA from raw or processed food of plant, animal or mixed origin by manual or automatic method 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 lysed in the presence of buffer preserving the integrity of even traces of DNA. Further, Proteinase K digests contaminating proteins, including 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 silica covered magnetic beads. Contaminants are efficiently removed in three 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.

- **GeneMAGNET line is based on the use of silica paramagnetic beads (Magnetic Beads) for selective binding of RNA and DNA. The use of specially designed binding and washing buffers enables the efficient purification of highly pure nucleic acids.**

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