EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMAGNET Plant DNA Purification Kit

Kit for purification of total DNA from plants, algae and fungi.

Cat. no. E3428

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Table of Contents

Kit content	96 preps E3428-01	Storage/Stability
Lyse Plant	63 ml	15-25°C
Wash P1	46 ml	15-25°C
Wash P2	70 ml	15-25°C
Wash P3	70 ml	15-25°C
Elution	30 ml	15-25°C
Magnetic Beads	1 ml	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.
- Disposable gloves, sterile pipette tips, sterile 1.5-2 ml tubes or 96-well plates with working volume at least 0.8 ml, vortex and tools for grinding the sample material eg. pestle and mortar, liquid nitrogen, mechanical homogenizer. We recommend Tissue Grinding Tool E0359-03, E0359-04.
- Isopropanol (100 %)

Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for isolation of DNA from different plant organs and tissues (leaves, seeds, fruits) as well as from fungi, algae and lichens. Use young plant parts, as they contain less polysacharides and polyphenols.

NOTE 2 · Maximum Sample Amount. One preparation enables purification of DNA from up to 50 mg wet weight tissue or 10 mg dry weight tissue (dried, lyophilized plant material).

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of Magnetic Beads which should be kept at 2-8°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 tissue.
   Grind plant or fungal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (up to 50 mg wet weight tissue or 10 mg dry weight tissue) in 2 ml Eppendorf tube and add 600 µl of **Lyse Plant** and vortex for 3 min.

   - *Tissue Grinding Tool E0359-03, E0359-04 is recommended for the homogenization. Insert plant fragment into the tube with grinding beads, add 600 µl Lyse Plant and grind by the pestle.*

   - *Add 3 µl of RNase A (E1350) during sample homogenization in order to obtain RNA-free DNA. This step is optional, without RNase A the obtained DNA isolate is suitable for molecular biology applications such as PCR or real time PCR.*

   - *To obtain high yield of DNA a tissue fragment should be thoroughly grinded to a fine powder.*

2. Centrifuge the lysate in a microcentrifuge for 3 min at 14 000 x g.

3. Carefully transfer 400 µl of the supernatant into a new tube.

   - *In some cases formed precipitates adhere loosely to the bottom of the tube. In such cases it is advised to transfer supernatant from only a few tubes simultaneously and continue centrifugation of remaining tubes.*

- *If it is impossible to transfer 400 µl of the supernatant into a new tube, reduce the starting weight of the sample or transfer as much liquid as possible and continue the protocol without changes in buffer volumes.*

4. Add 400 µl of isopropanol (100 %).

   - *Isopropanol is not included in this kit.*

5. Mix by vortexing for 30 s or pipetting 20 times.

6. 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 30 s. Incubate the sample at room temperature for 5 min.

   - *If working with 96-well plate format transfer the samples to the wells on the plate with working volume at least 0.8-1.0 ml.*

   - *If the sample volume is greater than the volume of the well or the magnet force is too low, transfer a part of the sample, place the plate on the magnet, after Magnetic Beads separation remove the supernatant. Continue the sample transfer to the 96-well plate until all the remaining part of the sample is processed.*

7. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets (3 min).

8. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 400 µl of **Wash P1** and mix by pipetting or vortexing for 10 s.

9. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

10. Remove and discard the supernatant by pipetting. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash P2** and mix by pipetting or vortexing for 10 s.

11. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

12. Remove and discard the supernatant by pipetting.

13. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash P3** and mix by pipetting or vortexing for 10 s.

14. Remove and discard the supernatant by pipetting. Leave the open tubes/plate in magnetic stand and air dry the beads for 15 min. **Wash P3** contains alcohol, make sure all the solution evaporates before proceding to the next step.

15. Add 100-200 µl **Elution** to the tube/well and mix by pipetting or vortexing. Incubate for 5 min at room temperature.

16. Separate the **Magnetic Beads** against the side of the tubes/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 or at -20°C.

GeneMagnet Plant DNA Purification Kit was tested on the following organisms

Plants:

Thale cress	*Arabidopsis thaliana*
Canola	*Brassica napus L. var. napus*
European yew (shoots)	*Taxus baccata*
Potatoe	*Solanum tuberosum*
Celeriac (leaves)	*Apium graveolens*
Parsley (leaves)	*Petroselinum crispum*
Dill (dried)	*Anethum graveolens*
Common flax (eeds)	*Linum usitatissimum*
Kiwi (fruit)	*Actinidia deliciosa*
Banana (fruit)	*Musa paradisiaca*
Apple (fruit)	*Malus domestica*
Sunflower (seeds)	*Helianthus annuus*
Walnut (nuts)	*Juglans regia*
Tomatoe	*Lyopersicon esculentum*

Fungi and lichens:

dried	*Boletus edulis*
Mun Fungi (dried)	*Auricularia auricula-judae*
mold	*Penicillium candidum*
mold	*Aspergillus sp.*
Lichen	*Lepraria incana*

Safety Information

Lyse Plant

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.

Wash B1

Danger

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 P2 / Wash P3

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 Plant DNA Purification Kit is designed for fast and easy purification of total DNA (genomic, mitochondrial and chloroplast) from a wide variety of plant, fungi and lichenes tissues.***

  Purified DNA is free of contaminants, such as: proteins, lipids, dyes, detergents, organic inhibitors of enzymatic reactions, buffers, salts, divalent cations, among others.

  Firstly, plant tissue is homogenized and lysed with the use of the lysis buffer, that also removes the contaminants from the sample. After centrifugation and isopropanol addition, DNA from the solution is bound to silica covered magnetic beads.

  The remaining contaminants are washed out during three washes steps. The eluted DNA is ready to use in molecular biology procedures.

- **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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