EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Plant & Fungi DNA Purification Kit

Universal kit for isolation of total DNA from plants, algae and fungi

Cat. no. E3595

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Table of Contents

Content	50 preps E3595-01	150 preps E3595-02	Storage/Stability
Buffer P	1.8 ml	5.4 ml	15-25°C
Lyse P	24 ml	72 ml	15-25°C
Lyse F	24 ml	72 ml	15-25°C
RNase A (10 mg/ml)	0.18 ml	0.54 ml	2-8°C
Proteinase K (20 mg/ml)	0.6 ml	1.8 ml	–20°C
AC	8 ml	23 ml	15-25°C
Sol P	21 ml	63 ml	15-25°C
Wash PX	60 ml	180 ml	15-25°C
Elution	18 ml	54 ml	15-25°C
DNA Binding Columns	50	3 x 50	15-25°C
Protocol	1	1	

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. To obtain greatest yield from leaves it is recommended to use youngest leaves possible, as they contain less polysacharides and polyphenols.

NOTE 2 · Maximum Sample Amount. One minicolumn enables purification of DNA from up to 100 mg wet weight tissue or 20 mg dry weight tissue (dried, lyophilized plant material). The maximum volume of the column reservoir is 650 µl. The maximum column binding capacity for DNA is 25 µg.

NOTE 3 · Kit Compounds Storage. Once the kit is unpacked, store components at room temperature, with the exception of RNase A and Proteinase K. RNase A should be kept at 2–8°C and Proteinase K at -20°C. In case of occasional buffer Lyse F ingredients precipitation, simply warm up in 37°C water bath, until clarified.

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 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

- For the basic protocol: Ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml collection tubes. Equipment for sample disruption and homogenization, depending on the method chosen: mortar and pestle and liquid nitrogen or handheld rotor-stator homogenizer, heating block capable of incubation at 65°C.
- Optional, in the case of DNA isolation from plant tissues rich in polysaccharides or oilseeds (see Appendix 1 on page 5 or Appendix 2 on page 7): chloroform, β-mercaptoethanol (14.3 M, β-ME) and Lyse CT buffer (EURx E0324) and a flat-bed vortex pad for shaking the sample.

Protocol

1. Apply 30 µl of activation **Buffer P** onto the spin-column (do not spin) and keep it at room temperature till transfering lysate to the spin-column (for best results at least 10 min).

   - *Addition of Buffer P 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.emove dirt and if possible the outer surface from the bone sample.*

2. 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 100 mg wet weight tissue or 20 mg dry weight tissue) in 2 ml Eppendorf tube and centrifuge the powder to the bottom of the tube. Add 400 µl of buffer **Lyse P** (plants, algae, lichens) or buffer **Lyse F** (fungi). Suspend the precipitate thoroughly.

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

3. Add 3 µl of **RNase A** and 10 µl of **Proteinase K**.

4. Mix by vortexing or several-fold inverting the tube and incubate the mixture for 30 min at 65°C (mix twice during incubation by inverting the tube).

5. Add 130 µl of buffer **AC**, mix thoroughly by inverting and incubate for 5 min on ice.

6. Centrifuge the lysate in a microcentrifuge for 10 min at 14 000 x g.

7. 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 adjust the volume of buffer Sol P and 96% ethanol proportionately in subsequent steps.*

8. Add 350 µl of buffer **Sol P**.

9. Add 250 µl of 96% ethanol. Mix thoroughly by several times inverting the tube.

10. Centrifuge for 1 min at 12 000 x g.

11. Transfer 600 µl of the lysate to the **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

12. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

    - *Continue centrifugation, if not all of the lysate passed through the column.*

13. Add 500 µl of **Wash PX** buffer and spin down at 11 000 x g for 1 min.
14. Remove spin-column, pour off supernatant, replace back spin-column.
15. Add 500 µl of **Wash PX** buffer and spin down at 11 000 x g for 1 min.
16. Remove spin-column, pour off supernatant, replace spin-column.
17. Spin down at 11 000 x g for 1 min to remove traces of the **Wash PX** buffer.
18. Place spin-column into new receiver tube (1.5–2 ml) and add 50–150 µl of **Elution** buffer 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.*

    - *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*

19. Incubate spin-column/receiver tube assembly for 2 min at room temperature.
20. Spin down at 11 000 x g for 1 min.
21. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/ manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

Appendix 1: DNA isolation from plant tissues rich in polysaccharides

NOTE 1 · This protocol is designed for isolation of genomic DNA from difficult plant tissues rich in starch, tannins or polyphenols.

NOTE 2 · To perform this isolation following components are necessary: chloroform, β-mercaptoethanol (14.3 M, β-ME) and Lyse CT buffer and a flat-bed vortex pad for shaking the sample. Lyse CT is not supplied with this kit, but is available as a separate product (Cat. no. E0324).

NOTE 3 · Add 50 µl β mercaptoethanol (β ME) per 10 ml Lyse CT buffer before use. Lyse CT is stable for 1 month after addition of β ME.

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

   - *Addition of Buffer P 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 tissue.
   Grind plant or fungal tissue under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (up to 100 mg wet weight tissue or 20 mg dry weight tissue) in 2 ml Eppendorf tube and centrifuge the powder to the bottom of the tube. Add 500 µl of buffer **Lyse CT**. Suspend the precipitate thoroughly.

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

3. Add 3 µl of **RNase A** and 10 µl of **Proteinase K**.

4. Mix by vortexing or several-fold inverting the tube and incubate the mixture for 30 min at 65°C (mix twice during incubation by inverting the tube).

5. Add 350 µl of chloroform to the lysate. Vortex for 10 15 min at room temperature.

6. Centrifuge for 10 min at 14 000 x g to separate the aqueous and organics phases

   - *Aqueous (upper) phase contains DNA.*

7. Carefully remove 400 µl aqueous (upper) phase without disturbing the lower phase, and transfer it to the new 1.5–2 ml Eppendorf tube.

8. Add 450 µl of buffer **Sol P**. Mix well by pipetting.

9. Transfer 600 µl of the lysate to the **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

10. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

    - *Continue centrifugation, if not all of the lysate passed through the column.*

11. Add 500 µl of buffer **Wash PX** to the spin-column and spin down at 11 000 x g for 1 min.

12. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.

13. Add 500 µl of buffer **Wash PX** to the spin-column and spin down at 11 000 x g for 1 min.

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

15. Place spin-column into new receiver tube (1.5–2 ml) and add 50–150 µl of **Elution** buffer to elute bound DNA.

    - *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.*

    - *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*

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

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

18. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/ manipulations. It can be stored at 2–8°C or (preferred) at -20°C.

Appendix 2: DNA isolation from oilseeds.

NOTE 1 · This protocol is designed for isolation of genomic DNA from seeds where the standard protocol described above does not produce the desired results. Usually this refers to oilseeds.

NOTE 2 · To perform this isolation following components are necessary: chloroform, β-mercaptoethanol (14.3 M, β-ME) and Lyse CT buffer and a flat-bed vortex pad for shaking the sample. Lyse CT is not supplied with this kit, but is available as a separate product (Cat. no. E0324).

NOTE 3 · Add 50 µl β-mercaptoethanol (β-ME) per 10 ml Lyse CT buffer before use. Lyse CT is stable for 1 month after addition of β-ME.

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

    - *Addition of Buffer P 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.*

2. Homogenization of tissue.
   Grind seeds under liquid nitrogen to a fine powder using previously cooled mortar and pestle. Place sample material (up to 100 mg ground seeds) in 2 ml Eppendorf tube and centrifuge the powder to the bottom of the tube. Add 500 µl of buffer **Lyse CT**. Suspend the precipitate thoroughly.

- *To obtain high yield of DNA a tissue fragment should be thoroughly grinded to a fine powder.*
- *In most cases, the best isolation results are obtained after homogenization by grinding seeds frozen in nitrogen.*

3. Add 3 µl of **RNase A** to the suspension of ground seeds.
4. Mix by vortexing or several-fold inverting the tube and incubate the mixture for 2 min at room temperature. Centrifuge for 2 min at maximum speed.
5. Carefully transfer of the supernatant (about 400 µl) into a new tube.

   - *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 adjust the volume to 400 µl with Lyse CT buffer.*

6. Add 350 µl of chloroform to the lysate. Vortex for 5 min at room temperature.
7. Centrifuge for 5 min at 14 000 x g to separate the aqueous and organics phases.

   - *Aqueous (upper) phase contains DNA.*

8. Carefully remove aqueous (upper) phase without disturbing the lower phase, and transfer it to the new 1.5-2 ml Eppendorf tube.
9. Add 10 µl of **Proteinase K**. Mix by vortexing or several-fold inverting the tube and incubate the mixture for 30 min at 65°C (mix twice during incubation by inverting the tube).
10. Add 450 µl of buffer **Sol P**. Mix well by pipetting.
11. Transfer 600 µl of the lysate to the **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.
12. Transfer the remaining mixture to the same **DNA binding spin-column** and centrifuge at 11 000 x g for 1 min. Remove the spin-column, pour off supernatant and place back into the receiver tube.

    - *Continue centrifugation, if not all of the lysate passed through the column.*

13. Add 500 µl of buffer **Wash PX** to the spin-column and spin down at 11 000 x g for 1 min.
14. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
15. Add 500 µl of buffer **Wash PX** to the spin-column and spin down at 11 000 x g for 1 min.
16. Spin down at 11 000 x g for 1 min to remove traces of the **Wash PX** buffer.
17. Place spin-column into new receiver tube (1.5-2 ml) and add 60-100 µl of **Elution** buffer to elute bound DNA.

- *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.*

- *In order to improve the efficiency of the elution genomic DNA from membrane, Elution buffer can be heated to a temperature of 80°C.*

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

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

20. Remove spin column, cap the receiver tube. Isolated DNA is ready for analysis/ manipulations. It can be stored at 2-8°C or (preferred) at -20°C.

Plant & Fungi DNA Purification Kit was tested on the following organisms:

Plant:

Spirogyrae (microscopic algae)	**Spirogyra**
Laminaria	**Laminaria spp.**
Bladder wrack	**Fucus vesiculosus**
Potato	**Solanum tuberosum**
Spruce	**Picea abies**
Cabbage	**Brassica spp.**
Larch	**Larix spp.**
Strawbery	**Fragaria x grandiflora**
Chives	**Allium cepa**
Rhododendron	**Rhododendron hort.**
Poa (grass)	**Poa spp.**
Rye	**Secale cerealis**
Mayze	**Zea mays**
Horse-chestnut	**Aesculus hippocastanum**
Maple	**Acer pseudoplatanus**
Malus	**Malus spp.**
Tomato (fruit and leaf)	**Lyopersicon esculentum**
Medicago (sprouts)	**Medicago L.**

Fungi and lichens:

Mold	**Penicillium candidum**
Mold	**Penicillium roqueforti**
	Tritirachium album
Reindeer lichen	**Cladonia Rangiferina**

Safety Information

Buffer P

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 P

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 P

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.
EUH208 Contains ethylenediammonium dichloride. May produce an allergic reaction.

Wash PX

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 Plant & Fungi DNA Purification Kit is designed for rapid 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: RNA, 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. 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 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 or water. Isolated DNA is ready for downstream applications without the need for ethanol precipitation.

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