EURx Molecular Biology Products

GeneMATRIX Plant & Fungi DNA Purification Kit

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

Cat. no. E3595

Version 5.1

April, 2008

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 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: One minicolumn enables purification of DNA from up to 100 mg wet weight tissue or 20 mg dry weight tissue (dried, lyophilized plant material).

Note 3: 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: All solutions should be kept tightly closed to avoid evaporation and resulting components concentration changes.

Note 5: The kit does not contain 96% ethanol, the reagent required during the DNA isolation procedure. This reagent needs to be provided by the user.

Protocol

1. Apply 40 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.

Note 1: Addition of Buffer P 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 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.

Note 1: 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 14000 rpm.
7. Carefully transfer 400 µl of the supernatant into a new tube.

Note 1: 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.

Note 2: 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 12000 rpm.
11. Transfer 600 µl of the supernatant to the spin-column placed in the collection tube.
12. Centrifuge for 1 min at 12000 rpm.

Note 1: Continue centrifugation at 14000 rpm if not all of the lysate passed through the column.

13. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
14. Transfer the remaining supernatant to the spin-column placed in the collection tube. Centrifuge again for 1 min at 12000 rpm to filtrate remains of the lysate through the resin.

Note 1: Continue centrifugation at 14000 rpm if not all of the lysate passed through the column.

15. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
16. Add 500 µl of buffer **Wash PX** to the spin-column and centrifuge for 1 min at 12000 rpm.
17. Take out the spin-column, discard flow-through and place back the spin-column in the collection tube.
18. Add 500 µl of buffer **Wash PX** to the spin-column and centrifuge for 2 min at 12000 rpm.
19. Place the spin-column in a new collection tube (1.5-2 ml) and add 100-200 µl of **Elution** buffer (10 mM Tris-HCl, pH 8.5) 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.

20. Incubate the spin-column/collection tube assembly for 3 min at room temperature.
21. Centrifuge for 1 min at 12000 rpm.

Optional:

22. Repeat elution once again as described in steps 19-21.

Note 1: This step improves DNA recovery from the column. A new collection tube can be used to prevent dilution of the first eluate or collection tube from step 19 can be reused to combine the eluates.
Note 2: More than 200 µl should not be used to elute into a single 1.5 ml microcentrifuge tube, as the spin-column will come into contact with the eluate, causing DNA contamination.

23. Discard the spin-column, cap the collection tube. Genomic 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 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.