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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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.

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 (see Appendix 1 page 6): 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.*

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.

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**

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

			ISOLATION OF DNA																		
			E3600	E3585	E3540	E3580	E3510	E3545	E3560	E3555	E3525	E3520	E3595	E3535	E3500	E3565	E3515	E3570	E3575	E3530	E3550	E3551
			MICELLULA DNA[2]	GRAM PLUS & YEAST GENOMIC DNA	AGAROSE – OUT DNA	BACTERIAL & YEAST GENOMIC DNA	BIO – TRACE DNA	BASIC DNA	BONE DNA	CELL CULTURE DNA	FOOD EXTRACT DNA	PCR / DNA CLEAN-UP	PLANT & FUNGI DNA	AGROBACTERIUM PLASMID DNA	PLASMID MINIPREP DNA	QUICK BLOOD DNA	SHORT DNA CLEAN-UP	SOIL DNA	STOOL DNA	SWAB-EXTRACT DNA	TISSUE DNA	TISSUE & BACTERIAL DNA
			AVAILABLE NUMBER OF ISOLATION (PREPS)																		
			50 150	25 100	50 150	50 150	25 100	50 150	25 50	50 150	25 100	50 150	50 150	50 150	50 150	50 150	25 100	50 100	50 100	25 100	50 150	50 150
DNA	GENOMIC	BACTERIA		●		●												●				●
		YEAST		●		●																
		CELL CULTURE								●											●	●
		PLANT											●									
		FUNGI											●									
		PLANT RICH IN POLYSACCHARIDES[1]											●									
		BLOOD														●						
		SOIL																●				
		STOOL																	●			
		SWAB																		●		
		ANIMAL TISSUES																			●	●
		FFPE TISSUE SECTIONS																			●	●
		RODENT TAILS																			●	●
		HAIR																			●	●
		INSECTS																			●	●
		URINE																			●	●
		BONE							●													
		BIOLOGICAL TRACES					●															
		FOOD									●											
	PLASMID	BACTERIA						●						●	●							
		YEAST				●																
	ISOLATION FROM AGAROSE GELS				●			●														
	PURIFICATION OF PCR PRODUCTS / DNA AFTER ENZYMATIC REACTIONS		●					●				●					●					

All kits contain buffers WASH in ready to use form

1. Additionally required lyse CT buffer (E0324)
2. Kit for creation of emulsions and subsequent DNA purification.

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SELECTION OF THE KITS DEPENDING ON THE TYPE OF ISOLATED MATERIAL

				DNA												RNA										
				GENOMIC												TOTAL RNA LONGER THAN 200 BASES					miRNA OR TOTAL RNA						
				BACTERIA	YEAST	CELL CULTURE	PLANT	FUNGI	PLANT RICH IN POLYSACCHARIDES	BLOOD/LEUKOCYTES	SOIL	STOOL	SEDIMENT	ANIMAL TISSUES		ANIMAL TISSUE	PLANT TISSUE	BACTERIA	YEAST	CELL CULTURE	ANIMAL TISSUE	PLANT TISSUE	CELL CULTURE	SOIL/STOOL/SEDIMENT	BACTERIA	YEAST	BLOOD/LEUKOCYTES	ON-COLUMN DNase DIGESTION
DNA/RNA PREPS	E3597	UNIVERSAL DNA/RNA /PROTEIN	25 100	●	●	●	●							●		●	●	●	●	●								●
	E3572	ENVIRONMENTAL DNA/RNA	25 100	●	●		●	●			●	●	●									●		●	●	●	
	E3750	DNA/RNA EXTRACOL [1]	25 100	●	●	●	●		●	●				●							●	●	●		●	●	●	

All kits contain buffers WASH in ready to use form

1. Kit contains phenol-based reagent for isolation DNA/RNA.

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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