EURx®
MOLECULAR
BIOLOGY
PRODUCTS

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GeneMATRIX Soil DNA Purification Kit

Kit for isolation of DNA from soil

Cat. no. E3570

DOMINIQUE DUTSCHER SAS

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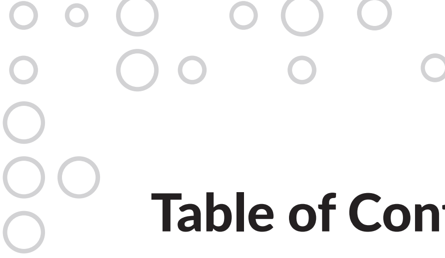

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Introductory Notes

NOTE 1 · Kit Specification. The kit is designed for the rapid isolation of pure, humic-free microbial DNA from environmental samples (soil, sediment, compost, manure). The isolated DNA allows for successful PCR amplification of: bacteria, fungi, protozoa, algae, etc.

NOTE 2 · Maximum Sample Portion. One minicolumn enables purification of DNA from up to 250 mg of dry soil or up to 100 mg wet soil. The maximum column binding capacity for DNA is 25 µg. The maximum volume of the column reservoir is 650 µl.

NOTE 3 · Kit Compounds Storage. The kit should be stored at room temperature, with the exception of PR buffer. PR buffer should be kept at 4°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.

Equipment and reagents to be supplied by the experimenter.

- Ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile pipet tips, sterile 1.5–2 ml collection tubes. Equipment for soil sample disruption and homogenization: a flat-bed vortex pad or cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.). Optional RNase A (10 mg/ml, we recommend using EURx RNase A, cat. no. E1350).

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Protocol

1. Apply 30 µl of activation **Buffer SL** onto the **DNA binding 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 SL 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. Add up to 250 mg of soil sample to the **Bead Tube**.

   - *The Bead Tube contains beads and buffer that enable dispersion of soil particles and cell lysis.*

   - *The kit is designed to process 0.25 g of soil. However, in some cases, it is necessary to optimize the soil sample weight. For more adsorbent soil types reduce soil sample weight to 0.1–0.15 g.*

   - *Optional, if RNA-free DNA is crucial for downstream applications, add 5 µl of RNase A (10 mg/ml). We recommend using EURx RNase A, cat. no. E1350.*

3. Mix by inverting the tube.

4. Add 60 µl **Lyse SL** buffer and invert several times the tube.

   - *The components of Lyse SL buffer can form precipitate in temperature below 20°C. In this case warm the buffer up in 37°C water bath and mix well, until it gets clarified.*

5. Secure **Bead Tubes** horizontally using a vortex adapter tube holder for the vortex or secure tubes horizontally on a flat-bed vortex pad with tape. Vortex at maximum speed for 10 min.

   - *Alternatively, a cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.) can be used, what enables to achieve higher yield. In this case processing time should be optimized.*

   - *If tubes are attached with a tape, you should be aware, that the tape may loosen. This may lead to inconsistent results or lower yields. Be sure that the tubes are tightly attached to the vortex.*

   - *In some cases better lysis yield can be achieved by freezing the sample. After the vortexing step, freeze the sample at -70°C. After thawing vortex sample another 5 min. The freezing/defrosting process can be repeated up to 3 times.*

6. Centrifuge the **Bead Tube** for 2 min at maximum speed and transfer 400 µl of the supernatant to the 2 ml microcentrifuge tube.

   - *Reduce the soil sample weight, when it is impossible to trasfer 400 µl of the supernatant.*

7. 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 humic substances, cell debris, and proteins.*

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8. Centrifuge for 1 min at maximum speed.

9. Transfer 600 µl of the supernatant to a new 2 ml microcentrifuge tube.

10. Add 600 µl **Sol SL** buffer.

11. Add 200 µl of 96% ethanol and mix thoroughly by vortexing or several times inverting.

12. Transfer 600 µl of the lysate to the **DNA binding spin-column** and centrifuge at 11 000 x g for 30 sec. Remove the spin-column, pour off supernatant and place back into the receiver tube.

13. Repeat step 12.

14. 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.

15. Add 500 µl **Wash SLX1** buffer to the spin-column and centrifuge for 1 min at 11 000 x g.

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

17. Add 500 µl **Wash SLX2** buffer to the spin-column and centrifuge for 1 min at 11 000 x g.

18. Spin down at 11 000 x g for 1 min to remove traces of the **Wash SLX2** buffer.

19. Place the spin-column in a new collection tube (1.5–2 ml) and add 50–100 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 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.*

   - *The following elution solutions can be used:*
     - *5–10 mM Tris-HCl buffer, pH 8.0–9.0.*
     - *0.5–1 x TE buffer, pH 8.0–9.0 (not recommended for DNA sequencing).*
     - *Other special application buffers can be used, if 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 2 min at room temperature.

21. Centrifuge the spin-column for 1 min at 11 000 x g.

22. Discard spin-column, cap the collection tube. DNA is ready for analysis/manipulation. It can be stored either at 2–8°C or at -20°C.

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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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GeneMATRIX is synonymous for a family of synthetic, new generation, nucleic acid binding membranes.

The GeneMATRIX membrane family has gained fame for two striking features: First, for their extraordinary high binding capacity, allowing to isolate nucleic acids with optimal yield. Second, for their remarkably high specificity. Even compounds of pronounced chemical similarity such as DNA, RNA and polysaccharides are easily differentiated amongst each other by the selectivity of these highly optimized matrices. This feature allows to isolate highly pure nucleic acids, that remain to work reliable even after being subjected to extended storage periods (years). Or, directly upon isolation, when used in subsequent molecular biology applications, such as: restriction digestion, dephosphorylation, kinasing, ligation, protein-DNA interaction studies, sequencing, blotting, in vitro translation, cDNA synthesis, hybridization, etc ...

We take great care to fine-tune all components and variables of the nucleic acid purification system towards each other – a multi-parameter optimization.

All matrices ship conveniently pre-packed in a ready-to-use spin-column format. Spin columns are specifically constructed for precise adjustment of liquid flow-through rate to optimal values. Novel binding and washing buffers are developed to take full advantage of GeneMATRIX unique capacity, resulting in isolation of biologically active, high-quality nucleic acids. And, last not least, a lot of time and efforts went into development of the various GeneMATRIces, thus providing a platform of unique chemical composition.

High and continuous reproducibility of matrix performance is always warranted, since component preparation as well as stringent quality control is entirely performed in-house at EURx Ltd.

Whatever your experience with nucleic acids isolation kits may look like, most likely you will encounter a difference with GeneMATRIX. And, we are so much convinced, you'll love it. Enjoy.

GeneMATRIX Soil DNA Purification Kit is designed for the rapid isolation of highly pure, humic-free microbial DNA from environmental samples: soil, sediment, compost, manure, etc. Purified DNA is free of contaminants, such as: humic substances, proteins, lipids, dyes, detergents, buffers, salts, divalent cations, etc.

Soil sample is added to a bead beating tube containing beads and lysis solution. The principal is to lyse the microorganisms in the soil by a combination of heat, detergent and mechanical force against the beads. Specialized solution is added to precipitate humic substances that strongly inhibit downstream applications. Optimized buffer and ethanol provide selective conditions for DNA binding to the DNA binding spin-columns. 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. Isolated DNA is ready for downstream applications without the need for the ethanol precipitation.

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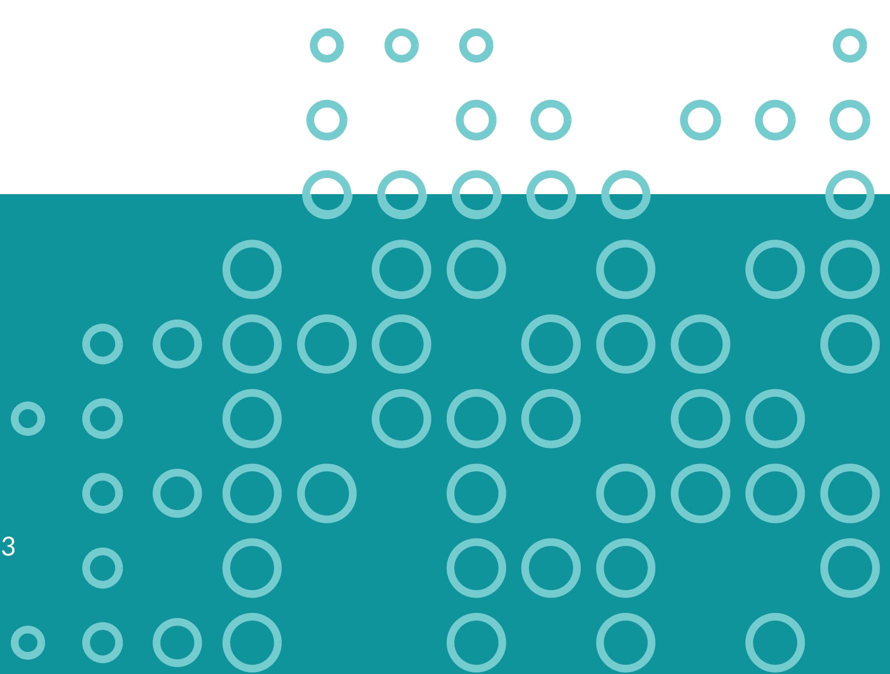

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