EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMATRIX Soil DNA Purification Kit

Kit for isolation of DNA from soil

Cat. no. E3570

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Content	50 preps E3570-01	100 preps E3570-02	Storage/Stability
Buffer SL	1.8 ml	3.6 ml	15-25°C
Lyse SL	3.6 ml	7.2 ml	15-25°C
PR	24 ml	48 ml	2-8°C
Sol SL	36 ml	72 ml	15-25°C
Wash SLX1	30 ml	60 ml	15-25°C
Wash SLX2	30 ml	60 ml	15-25°C
Elution	6 ml	12 ml	15-25°C
DNA Binding Columns	50	2 x 50	15-25°C
Bead Tube	50	2 x 50	15-25°C
Protocol	1	1	

Equipment and reagents to be supplied by the user

- Ethanol [96–100% v/v], microcentrifuge, disposable gloves, sterile pipette 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).

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 and filtered water samples). The isolated DNA allows for successful PCR amplification of: bacteria, fungi, protozoa, algae, nematodes, 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.

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.

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.*

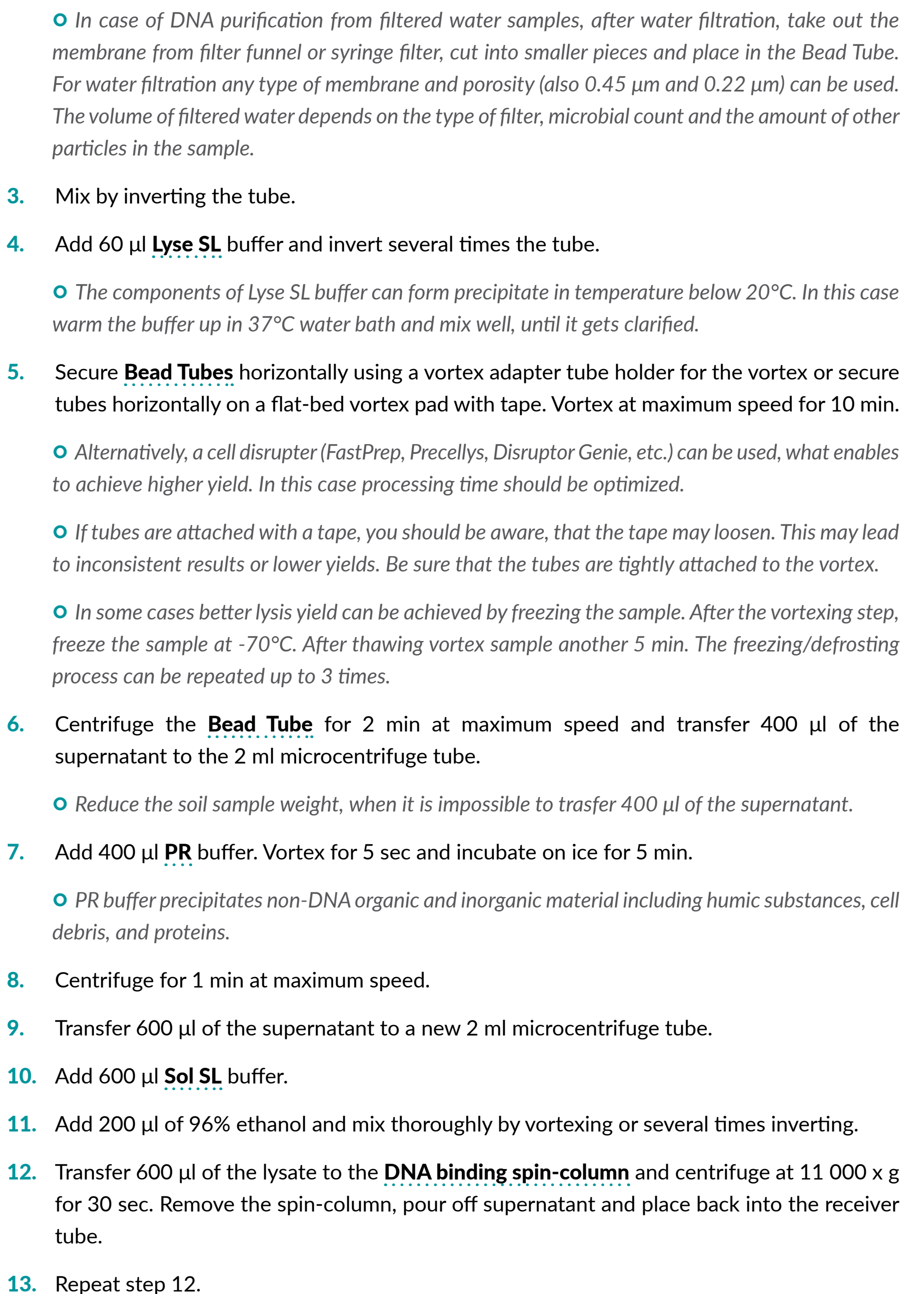
   - *The membrane activation should be done before starting the isolation procedure.*

2. Add up to 250 mg of soil sample to the **Bead Tube**.

   - *The Bead Tube contains glass beads and 750 µl of 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.*



- *In case of DNA purification from filtered water samples, after water filtration, take out the membrane from filter funnel or syringe filter, cut into smaller pieces and place in the Bead Tube. For water filtration any type of membrane and porosity (also 0.45 µm and 0.22 µm) can be used. The volume of filtered water depends on the type of filter, microbial count and the amount of other particles in the sample.*

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.*

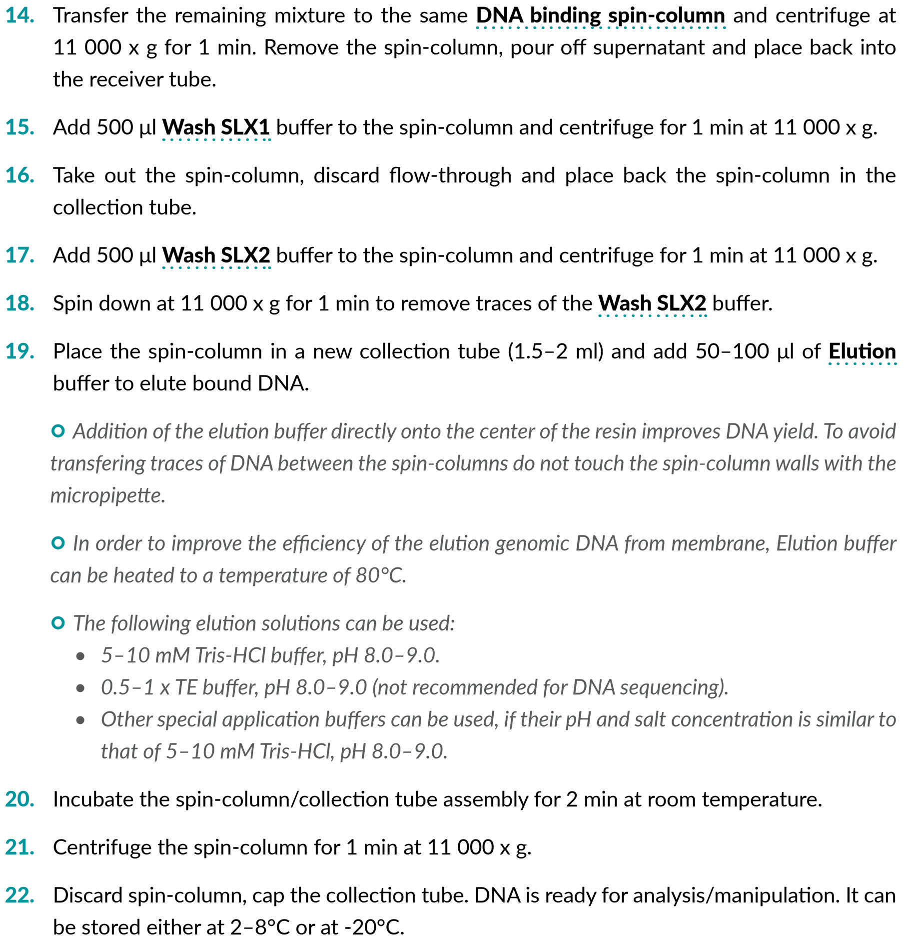
6. Centrifuge the **Bead Tube** for 2 min at maximum speed and transfer 400 µl of the supernatant to the 2 ml microcentrifuge tube.

   - *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.*

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 micropipette.

   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.

Safety Information

Buffer SL

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.

Sol SL

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 SLX1

Warning

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 SLX2

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 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 and water samples. 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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