EURx® MOLECULAR BIOLOGY PRODUCTS

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GeneMAGNET Soil DNA Purification Kit

Kit for isolation of DNA from environmental samples
(soil, sand, sediment, compost, manure and filtered water samples)

Cat. no. E3421

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Kit content	96 preps Exxxx-01	Storage/Stability
Lyse SL	7.2 ml	15-25°C
PR	48 ml	2-8°C
Wash S1	48 ml	15-25°C
Wash S2	130 ml	15-25°C
Elution	24 ml	15-25°C
Magnetic Beads	25 ml	2-8°C
Bead Tube	2 x 50	15-25°C
Protocol	1	

Equipment and reagents to be supplied by the user

- Magnetic stand E0361 for 16 tubes, E0362 for 24 tubes, E0363 for 96-well plate. To be purchased separately.
- Disposable gloves, sterile pipette tips, sterile 1.5–2 ml collection tubes or 96-well plates.
- Equipment for soil sample disruption and homogenization: a flat-bed vortex pad or cell disrupter (FastPrep, Precellys, Disruptor Genie, etc.).
- Ethanol [96–100% v/v]. 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: Gram+ and Gram- bacteria, fungi, protozoa, algae, nematodes, plants and animals.

NOTE 2 · Maximum Sample Portion. One preparation enables purification of DNA from up to 250 mg of dry soil, 100 mg wet soil or 1 cm2 of membrane after water filtration. Sample volume can be increased or decreased, but the amount of DNA binding buffers needs to be scaled up or down. For example: to 300 µl of sample after precipitation with PR buffer add 125 µl of magnetic beads. The amount of washes and elution buffer can be adjusted proportionally. Note the limit of the vial maximal volume.

NOTE 3 · Kit Compounds Storage. Upon the arrival, the kit should be stored at room temperature, with the exception of PR buffer and magnetic beads, which should be kept at 2-8 °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.

Protocol

1. Add up to 250 mg of soil sample or 100 mg feces 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 250 mg 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 100–150 mg or 100 mg feces. In case of a very dry stool samples, especially of herbivores, reduce the sample weight to 50 mg.*

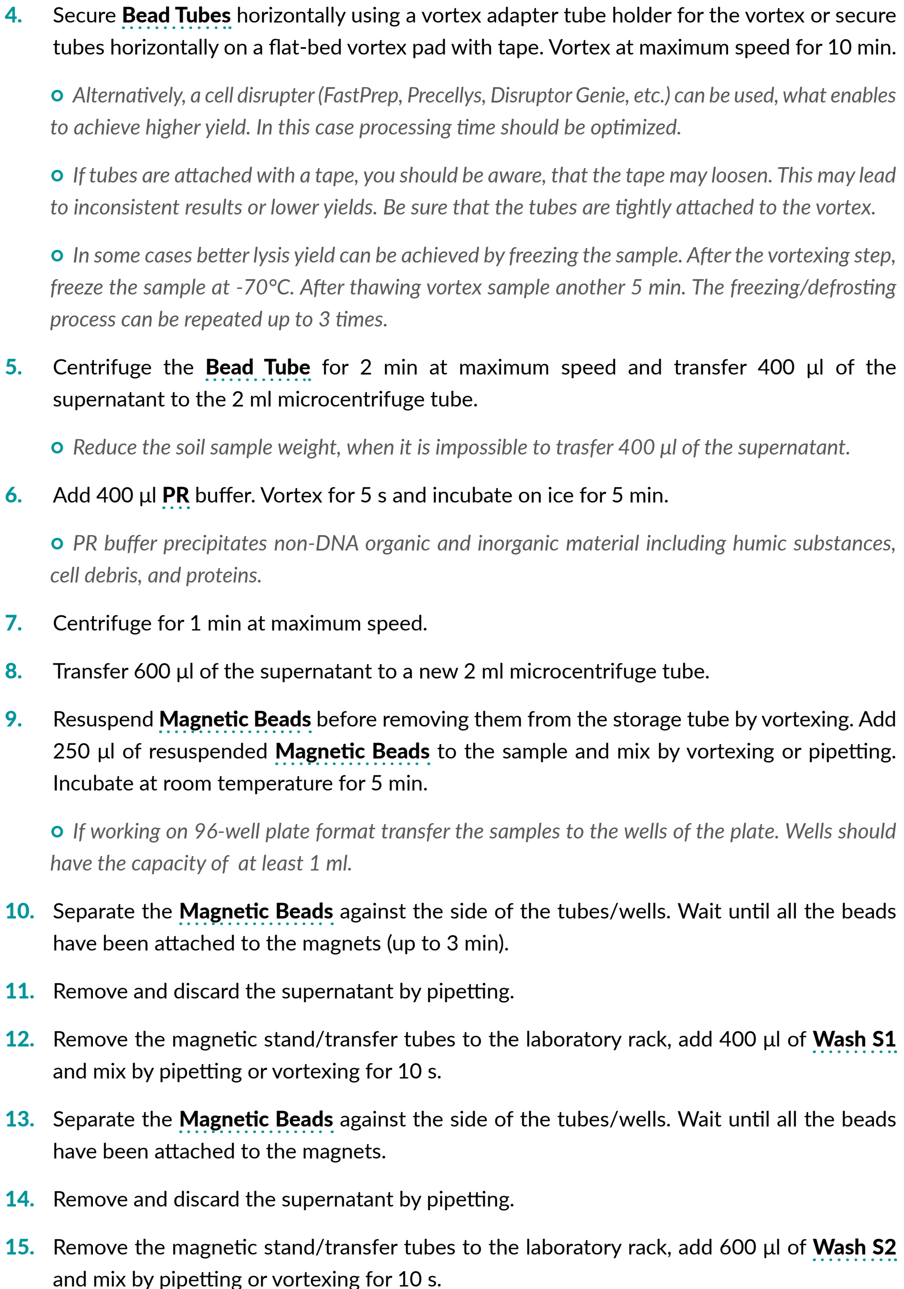
   - *Optional, if RNA-free DNA is crucial for downstream applications, add 5 µl of RNase A (10 mg/ml). We recommend using EURx RNase A, cat. no. E1350.*

   - *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.*

2. Mix by inverting the tube.

3. 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.*



4. 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.*

5. 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.*

6. Add 400 µl **PR** buffer. Vortex for 5 s and incubate on ice for 5 min.

   - *PR buffer precipitates non-DNA organic and inorganic material including humic substances, cell debris, and proteins.*

7. Centrifuge for 1 min at maximum speed.

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

9. Resuspend **Magnetic Beads** before removing them from the storage tube by vortexing. Add 250 µl of resuspended **Magnetic Beads** to the sample and mix by vortexing or pipetting. Incubate at room temperature for 5 min.

   - *If working on 96-well plate format transfer the samples to the wells of the plate. Wells should have the capacity of at least 1 ml.*

10. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets (up to 3 min).

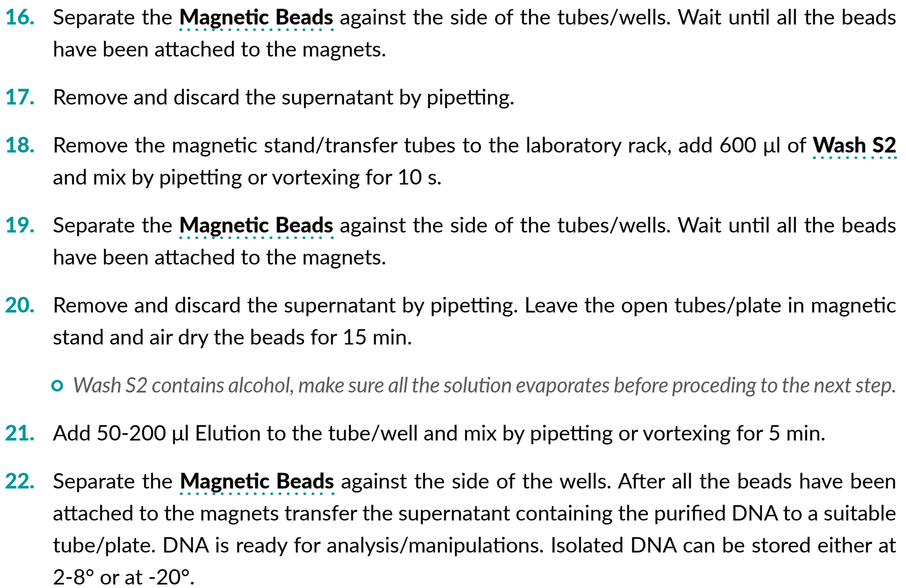
11. Remove and discard the supernatant by pipetting.

12. Remove the magnetic stand/transfer tubes to the laboratory rack, add 400 µl of **Wash S1** and mix by pipetting or vortexing for 10 s.

13. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.

14. Remove and discard the supernatant by pipetting.

15. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash S2** and mix by pipetting or vortexing for 10 s.



16. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
17. Remove and discard the supernatant by pipetting.
18. Remove the magnetic stand/transfer tubes to the laboratory rack, add 600 µl of **Wash S2** and mix by pipetting or vortexing for 10 s.
19. Separate the **Magnetic Beads** against the side of the tubes/wells. Wait until all the beads have been attached to the magnets.
20. Remove and discard the supernatant by pipetting. Leave the open tubes/plate in magnetic stand and air dry the beads for 15 min.
    - *Wash S2 contains alcohol, make sure all the solution evaporates before proceding to the next step.*
21. Add 50-200 µl Elution to the tube/well and mix by pipetting or vortexing for 5 min.
22. Separate the **Magnetic Beads** against the side of the wells. After all the beads have been attached to the magnets transfer the supernatant containing the purified DNA to a suitable tube/plate. DNA is ready for analysis/manipulations. Isolated DNA can be stored either at 2-8° or at -20°.

Safety Information

Wash S1

Danger

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.

P261 Avoid breathing dust/fume/gas/mist/ 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.

P337+**P313** If eye irritation persists: Get medical advice/ attention.

Wash S2

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.

P337+**P313** If eye irritation persists: Get medical advice/ attention.

P403+**P235** Store in a well-ventilated place. Keep cool.

- ***GeneMAGNET Soil DNA Purification Kit is magnetic beads based kit for the isolation of total 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 or salts.***

  The sample is added to a tube containing glass beads and the lysis buffer. DNA is released into the solution during shaking. Next, humic substances that strongly inhibit downstream applications, are precipitated by PR buffer. DNA bind to magnetic beads suspended in the binding buffer. Contaminants are removed from the samples in three wash steps. High-quality cellular DNA is then eluted in low salt buffer. Isolated DNA is ready for downstream applications without the need for the ethanol precipitation.

- **GeneMAGNET line is based on the use of silica paramagnetic beads (Magnetic Beads) for selective binding of RNA and DNA. The use of specially designed binding and washing buffers enables the efficient purification of highly pure nucleic acids.**

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