DANAGENE MICROBIOME SOIL DNA KIT

Ref. 0621 50 PREPS

1. INTRODUCTION

The DANAGENE MICROBIOME Soil DNA kit has been designed for a fast and efficient purification of **microbial DNA from environmental samples like soil samples**

In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Inhibitors are eliminated by precipitation using a proprietary cleanup buffer. The sample is then applied to a microspin column and the DNA that is bound to the column undergoes a single wash step before elution.

Features:

- **Designed for a fast and easy purification microbial DNA from different types of soil samples.**
- **Optimized lysis method-combination of heat, chemical and mechanical lysis via bead-based homogenization enables isolation of DNA from yeast, fungi, Gram-negative and Gram-positive bacteria.**
- **Eliminates inhibitory substances as humic substances and others inhibitors.**
- **No phenol/chloroform extraction or ethanol precipitation is necessary.**

Aplications:

- **Microbiome analysis**
- **PCR applications.**
- **RFLP analysis.**
- **Patogen typing.**
- **Mutation analysis.**

2. COMPONENTS KIT

	50 preps	Storage
Lysis Buffer Soil	**30 ml**	Room temperature
Enhancer Buffer	**5 ml**	Room temperature
EC Buffer	**8 ml**	Room temperature
Binding Buffer Soil	**50 ml**	Room temperature
Wash Buffer *	**10 ml**	Room temperature
Elution Buffer	**10 ml**	Room temperature
Bead Microtubrs	**50 unidades**	Room temperature
Proteinase K *	**30 mg**	-20ºC
Microbial DNA Columns	**50 unidades**	Room temperature
Collection Tubes	**100 unidades**	Room temperature

(*) These solutions must be prepared as indicated in the Preliminary Preparations section of the protocol.

PRECAUTIONS: The Lysis Buffer Soil and Desinhibition Buffer contains guanidium salts, and should be handled with care. Guanidinium salts form highly reactive compounds when combined bleach.

2.1 Equipment and additional reagents required

- Microcentrifuge.
- Microcentrifuge tubes, DNase-free, 1.5 mL and 2.0 ml
- Ethanol 100%.
- Heat block, dry bath, or water bath (65°C).
- For vortex bead homogenization: hands-free adapter for vortex mixer, with horizontal tube orientation, we recommend the Vortex Genie 2.
- (Optional) alternative to vortex bead homogenization: Bead mill homogenizer.

3. PROTOCOL

3.1 Preliminary Preparations

- Dissolve the proteinase K in **1.3 ml of nuclease-free water** and store at –20ºC. It is recommended to do several aliquots to avoid many thaw/freeze cycles. At this temperature it is stable for 1 year.

- **Add 40 ml of Ethanol 100 %** to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.

- Pre-heat the Elution Buffer at 70ºC.

3.2 General Remarks

Amount of starting material

Usually a reduction of starting material also helps to improve the lysis efficiency and to increase the purity of the DNA.

Very dry material can soak up large volumes of lysis and enhancer buffer. In this case, either reduce the amount of sample material or add additional lysis and enhancer buffer.

If possible remove foreign material like leaves, stones, or twigs (e.g., by sieving) as well as excess of water (e.g., by discarding the supernatant after spinning down sediment samples).

Sample Handly

Collect samples according to your laboratory guidelines and experimental needs.
Ensure that samples are mixed thoroughly with Lysis Buffer and Enhancer Buffer to create a homogenous sample.
One way to ensure thorough mixing is to vortex the tube with the cap down.

Sample Lysis

The procedure is optimized by using "beads" in a vortex with horizontal agitation (Vortex Genie 2 or similar). Make sure that the vortex adapter allows horizontal agitation; **This is a cost-effective method for recovery of high-quality microbial DNA.**

Adapters with a vertical tube orientation my not agitate properly.

You can use "Bead mil" homogenizers such as FastPrep, Precellys and others but following the manufacturer's instructions to optimize the lysis of the sample. IMPORTANT: Many modern disruption devices can cause very high energy input in bead tubes. Depending on bead tube type and content (beads, liquid volume, sample type), especially high frequency of shaking and / or long shaking duration can cause breaking up of the bead tubes! **It is the responsibility of the user to perform initial stability test for the used bead tubes under the conditions used!** Perform initial test with water instead of lysis buffer and moderate machine setting (low frequency, short time) in order to avoid spillage of chaotropic lysis buffer in case of tube breakage.

3.3 Protocol for DNA extraction from 250 mg of environmental samples

1. Weigh **250 mg of soil sample** in a 2.0 mL **bead microtube** and add **600 µL of Lysis Buffer Soil.** Vortex.

Depending on the nature of the soil it may be necessary to increase the Lysis Buffer Soil and Enhancer Buffer proportionally with the aim of recovering 400 µl of supernatant in point 5. You can order additional buffers.

2. Add **100 µL of Enhancer Buffer**. Mix well by shaking gently the microtube.
3. **Add 25 µl of Proteinase K . Incubate at 65ºC for 10 minutes.**
4. **Homogenize** by bead beating for **10 minutes** at maximum speed on the Vortex Genie 2 or similar using a **horizontal adapter.**

The lysis time should be as short as necessary to avoid shearing of DNA and to minimize the release of humic acids. **Depending on the sample**, however, it might be advantageous to increase the lysis time to 10, 20, or 30 min.

5. **Centrifuge at 14.000 rpm for 5 minutes**. Transfer up to **400 µL of the supernatant** to a clean microcentrifuge tube.
   IMPORTANT: A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.
6. Add **150 µl EC Buffer.Vortex.** Incubate at 0-4ºC for 5 minutes.
7. **Centrifuge at 14,000 rpm for 3 minutes.** Transfer up to **500 µl of supernatant** in a new 1.5 ml microtube avoiding touching the pellet.
8. Add **900 µl of Binding Buffer Soil** and vortex briefly.
9. Load **700 µl** mixture sample into reservoir of a combined Microbial DNA column–collection tube assembly. **Centrifuge at 8.000 rpm for 30 seconds.** Remove the collection tube.
10. Discard the flow-through, and repeat step 9 with the remaining sample mixture.

Ensure that the entire sample mixture has passed into the collection tube by inspecting the column. If sample remains in the column, centrifuge again at 14,000 rpm for 1 minute.

11. Add **700 µl of Wash Buffer. Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

12. **Dry silica membrane.** Centrifuge at 14.000 rpm for **3 minutes.**

13. Place the Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **50-100 µL Pre-heat the Elution Buffer** at 70ºC. Incubate **at room temperature** for **2 minutes**.

14. **Centrifuge** the spin column-tube assembly **at 14.000 rpm for 1 minute**, then discard the column. The purified DNA is in the tube.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

Due to the great environmental samples variety that can be treated, it becomes difficult to generalize possible problems and answers. For this reason, we recommend to contact **DANAGEN-BIOTED** Laboratory Technical Service for any question regarding the protocols, specific soil samples or any problem you may have during the process.
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