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DANAGENE MICROBIOME FECAL DNA KIT

Ref. 0620 50 PREPS

1. INTRODUCTION

The DANAGENE Microbiome Fecal DNA kit is designed for the efficient isolation of microbial DNA from:

1. **up to 250 mg fresh and frozen human or animal stool samples.**
2. **250 µl preserved stool sample stabilized in DANASTOOL Sample Collection MICROBIOME Kit.**
3. **400 µl preserved stool sample stabilized in DANASWAB Sample Collection MICROBIOME Kit.**

Our **New DANAGENE Microbiome Fecal DNA kit** is even more effective than our original Microbiome Fecal technology and is designed to isolate high yields of pure **microbial DNA from stool samples for microbiome and metagenomic analysis.**

The kit features a novel **Microbial DNA Column** and optimized chemistry for a more efficient removal of PCR inhibitors (such as polysaccharides, heme compounds and bile salts) using a novel **Microbiome Lysis Buffer and Microbiome Precipitation Buffer**

In this procedure, the microorganisms are efficiently lysed by a combination of heat, chemical and mechanical disruption with specialized beads. Proteins and Inhibitors are eliminated by precipitation using a new proprietary inhibitor removal buffer and subsequently pelleted by centrifugation together with the beads and undissolved sample material. The purified lysate is mixed with the Binding Buffer and then applied to a Microbial DNA column. The DNA that is bound to the column undergoes a two wash step. After a drying step, ready to use DNA for NGS, PCR and other downstream applications can be eluted with Elution Buffer (5 mM Tris/HCl, pH 8.5).

2. COMPONENTS KIT

	50 preps	Storage
Microbiome Lysis Buffer	**60 ml**	Room temperature
Microbiome Precipitation Buffer	**12 ml**	4ºC
Binding Buffer	**45 ml**	Room temperature
Desinibition Buffer	**28 ml**	Room temperature
Wash Buffer	**45 ml**	Room temperature
Elution Buffer	**12 ml**	Room temperature
Bead Microtubes	**50 units**	Room temperature
Proteinase K *	**30 mg**	-20ºC
Microbial DNA Columns	**50 units**	Room temperature
Collection Tubes	**150 units**	Room temperature

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

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

Intended Use

All DANAGENE products are intended for molecular biology applications. These products are not intended for the diagnosis, prevention, or treatment of a disease.

2.1 Equipment and additional reagents required

Reagents
• 96 – 100 % ethanol

Consumables
• 1.5 mL microcentrifuge tubes
• Disposable pipette tips

Equipment
• Manual pipettors (50–1000 µl)
• Heat block, dry bath, or water bath (70°C)
• Centrifuge for microcentrifuge tubes
• Equipment for sample disruption and homogenization (see section 3.2). Vortex-Genie® 2
• Personal protection equipment (e.g., lab coat, gloves, goggles)

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.

- **Pre-heat the Elution Buffer at 70ºC.**

3.2 General Remarks

Amount of starting material

DANAGENE MICROBIOME FECAL DNA kit is optimized for processing 180–220 mg of human stool. Very dry material can soak up large volumes of lysis. In this case, either reduce the amount of sample material or add additional lysis buffer.

The kit contains sufficient Microbiome Lysis Buffer for 50 samples using up to 1200 µl per sample. However, fill the Bead Tube (including the beads) to ensure sufficient head space for an efficient mechanical disruption**.**

For stool samples from animals, lowering the sample amount may lead to better results. Very dry stool samples like rabbit or mouse feces may absorb lysis buffer, resulting in an insufficient sample volume after the first centrifugation step. In these cases, it is recommended to reduce the amount of stool material to e.g., 60 – 80 mg and to increase the total lysis volume to 1 -1.2 ml.

For difficult stool samples like lipid, polysaccharide, or protein rich stool, a reduction of starting material might also improve the lysis efficiency and the purity of the DNA. It is recommended in such cases to start the extraction with 60 – 80 mg sample material.

Human stool samples may also contain undigested food matter (e.g., crop or fruit husks, undigested seeds). These particles should not be transferred to the Bead Tubes.

Sample Homogenization

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 may not agitate properly.

Processing time will vary based on sample input and bead beater. Times may be as little as 5 minutes when using high-speed cell disrupters (FastPrep) or as long 10-20 minutes when using lower speeds (Vortex-Genie® 2).

The DANAGENE MICROBIOME FECAL DNA kit does not require homogenization using a high-velocity bead beater. However, if the microorganism of interest requires stronger homogenization than provided by a vortex, or if using a bead beater is desired **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 microbial DNA extraction from fresh or frozen stool samples

1. Add **180-220 mg of human stool sample** in a 2.0 ml **bead microtube** and add **850 µL of Microbiome Lysis Buffer.** Vortex briefly to mix.

Depending on the nature of the stool, it may be necessary to increase the Microbiome Lysis Buffer proportionally with the aim of recovering 500-600 µl of supernatant in point 4 (dry samples).

The kit contains sufficient Microbiome Lysis Buffer for 50 samples using 1200 µl per sample.

*For stool samples from animals, lowering the sample amount may lead to better results. Very dry stool samples like rabbit or mouse feces may absorb lysis buffer, resulting in an insufficient sample volume after the first centrifugation step. In these cases, it is recommended to reduce the amount of stool material to e.g., 60 – 80 mg and to increase the total lysis volume to 1 -1.2 ml.

Stool Sample	Fresh or Frozen	Preserved in DANASTOOL	Preserved in DANASWAB
Amount starting material	**180-220 mg***	**250 µl**	**400 µl**

2. **Add 25 µl of Proteinase K. Incubate at 70ºC for 10 minutes.**
3. Homogenize samples thoroughly using one of the explained methods.

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. ***Depending on the sample****, however, it might be advantageous to increase the lysis time to 10, 20, or 30 min.*

4. **Centrifuge at 16.500 x g for 2 minutes**. Transfer **600 µl supernatant** to a clean 1.5 ml microcentrifuge tube.
   IMPORTANT: Expect 500-600 µl of supernatant**.** A layer of debris may be present on top of the bead pellet. Avoid transfer of this debris with the supernatant.
5. Add **200 µl Microbiome Precipitation Buffer. Vortex.**
6. **Centrifuge at 16.500 x g for 2 minutes.** Transfer **600 µl of supernatant** in a new 1.5 ml microtube avoiding touching the pellet.
7. Add **900 µl of Binding Buffer** and vortex briefly.
8. Load **750 µl** mixture sample into reservoir of a combined Microbial DNA column–collection tube assembly. **Centrifuge at 15.000 x g for 1 minute.**
9. Discard the flow-through and place the Spin Column back into the same 2 ml Collection Tube, repeat step 8 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 15.000 x g for 1 minute.

10. Carefully place the Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the Spin Column.
11. Add **500 µl of Desinhibition Buffer. Centrifuge at 15.000 x g for 1 minute.** Remove the collection tube.

12. Add **700 µl of Wash Buffer. Centrifuge at 16.000 x g for 1 minute.** Remove the collection tube.

13. Carefully place the Spin Column into a clean 2 ml Collection Tube (provided). Avoid splashing any flow-through onto the Spin Column.

14. **Dry silica membrane.** Centrifuge at 16.500 x g for **2 minutes.**

15. Place the Microbial DNA Column into a 1.5 mL nuclease-free tube (not provided) and add **100-200 µL Pre-heat the Elution Buffer** at 70ºC o the centre of the white filter membrane. Incubate **at room temperature** for **2 minutes**.

16. **Centrifuge** the spin column-tube assembly **at 15.000 x g for 1 minute**, then discard the column. The DNA is now ready for downstream applications.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

Due to the great stool 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 or any problem you may have during the process.
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