November 2023

DANAGENE MICROBIOME DANASTOOL KIT

Ref.0620.50DANASTOOL 50 PREPS
Ref.0620.250DANASTOOL 250 PREPS

1. INTRODUCTION

The DANAGENE MICROBIOME Fecal DNA kit has been designed for a fast and efficient purification of **microbial DNA** from:

a) Stool homogenate from 0.50-1.0 gr stool and stabilized in 8 ml DANASTOOL .

2. COMPONENTS KIT

	50 PREPS	250 PREPS	Almacenamiento
EC Buffer	**10 ml**	**52 ml**	Temperatura ambiente
Binding Buffer	**15 ml**	**65 ml**	Temperatura ambiente
Desinhibition Buffer*	**18 ml**	**82.50 ml**	Temperatura ambiente
Wash Buffer *	**10 ml**	**50 ml**	Temperatura ambiente
Elution Buffer	**10 ml**	**55 ml**	Temperatura ambiente
Bead Microtubes	**50 unidades**	**250 unidades**	Temperatura ambiente
Proteinasa K*	**30 mg**	**5 x 30 mg**	-20ºC
Microbial DNA Columns	**50 unidades**	**250 unidades**	Temperatura ambiente
Collection Tubes	**100 unidades**	**500 unidades**	Temperatura ambiente

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

PRECAUTIONS: The Desinhibition 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.

- **Add 10 ml (50 PREPS) or 50 ml (250 PREPS) of Ethanol 100 %** to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation.

- **Add 40 ml (50 PREPS) or 200 ml (250 PREPS) of Ethanol 100 %** to the Wash Buffer. Keep the container closed to avoid the ethanol evaporation.

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

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;

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.

3.2 Protocol for DNA extraction from stool samples STABILIZED into DANASTOOL Sample Collection Kit

SAMPLE COLLECTION:

1. Using the spoon attached to the lid take stool sample from 2-3 different sites and transfer the samples to the preservative fluid. Approximately half a spoon per take is enough. The liquid allows to stabilize 0.5-1.0 gr of sample. **It is recommended:** Depending on the size and characteristics of the stool sample, mixing the sample completely to generate a homogeneous sample in this way will not be necessary to take sample from 3 different sites of the sample.
2. Close the container well and shake to achieve the homogenization of the fecal matter with the preservative liquid. This will be achieved more quickly depending on the consistency of the fecal matter, for hard consistencies this can be achieved by helping with the spoon, or stirring the tubes every day until the day of extraction.
3. Label the stool tube with your full name and date of collection.
4. Send the sample to the laboratory, sending it to room temperature. The sample is stable for several months at room temperature (15-25ºC) and indefinitely at -20 o -80ºC.
5. For extraction, several different methods can be used, **the use of our DANAGENE MICROBIOME DANASTOOL Kit is recommended.**

MICROBIAL DNA EXTRACTION:

1. Transfer **1.0 ml of stabilized stool sample** to a 2.0 ml **bead microtube** containing particles.

   Before transferring the sample, make sure that the sample is completely homogenized, for the transfer it is recommended to cut a 1000 µl tip to make the mouth wider and take 2 x 500 µl, mixing the sample well with the micropipette.

2. Resuspend the sample with simple shaking of the microtube or micropipette. No vortex. **Incubate at 70 ° C for 10 minutes.**

3. **Homogenize** by bead beating for 10 minutes at maximum speed on the Vortex Genie 2 or similar using a **horizontal adapter.**

4. **Centrifuge at 14.000 rpm for 5 minutes**. Transfer up to **600 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.

5. Add **200 µl EC Buffer Vortex.** Incubate at 4ºC for 5 minutes

6. **Centrifuge at 14.000 rpm for 5 minutes**. A pellet will appear and in the surface a layer of fat, to introduce the pipette tip crossing this superficial layer of fat, only trying to pick up **500 µl of supernatant** that it is the transparent liquid with color (to avoid to catch pellet and superficial layer) and to place in a 1.5 ml microtube.

7. **Add 25 µl of Proteinase K. Incubate at 70ºC for 10 minutes.**

8. Add **250 µl of Binding Buffer** and vortex briefly.

9. Add the lysate into reservoir of a combined Microbial DNA Column–collection tube assembly. **Centrifuge at 10.000 rpm for 60 seconds.** Remove the collection tube.

10. Place the Microbial DNA column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 12.000 rpm for 1 minute.** Discard the flow-through.

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 **200 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

For any doubts or additional questions about the protocol, please contact the technical service of DANAGEN-BIOTED S.L info@danagen.es