DANAGENE MICROBIOME SWAB DNA KIT

Ref.0625 50 Preps

1. INTRODUCTION

The DANAGENE MICROBIOME Swab DNA kit has been designed for a fast and efficient purification of **microbial DNA from preserved samples using our DANASWAB Sample Collection MICROBIOME Kit for microbiome analysis.**

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.

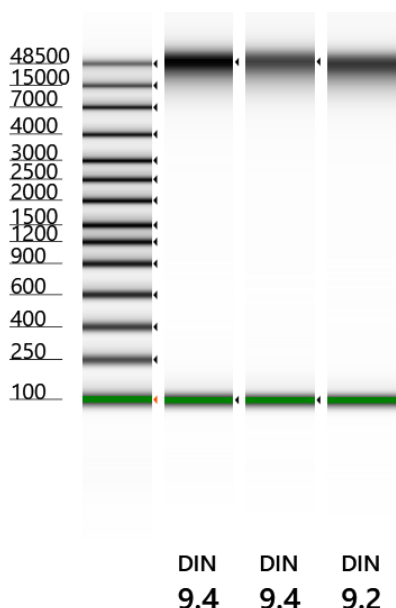
Features:

- **Designed for a fast and easy purification microbial DNA from preserved samples using our DANASWAB Sample Collection MICROBIOME Kit.**
- **Optimized lysis method-combination of heat, chemical and mechanical lysis via bead-based homogenization enables isolation of microbial DNA for microbiome analysis.**
- **Eliminates inhibitory substances.**
- **No phenol/chloroform extraction or ethanol precipitation is necessary.**

Applications:

- **Microbiome analysis**
- **PCR applications.**
- **RFLP analysis.**
- **Pathogen typing.**

High-Quality DNA



DNA was isolated from aliquots of 800µl of preserved feces samples with our DANASWAB Sample Collection MICROBIOME kit. These samples were preserved at room temperature for 30 days. Isolation was carried out on day 1, day 15 and day 30 using our **DANAGENE MICROBIOME Swab DNA Kit**. Quality was assessed using the Agilent 4150 TapeStation.

2. COMPONENTS KIT

	50 preps	Storage
Microbiome Lysis Buffer	**45 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	**6 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

- 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 mill" 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 samples preserved in DANASWAB Sample Collection MICROBIOME Kit

IMPORTANT: Prior to purification of DNA wait at least 24 hours and vortex vigorously to properly homogenize the preserved sample.

1. Add **850 µL of Microbiome Lysis Buffer + 400 µL of MICROBIOME Stabilization Solution with preserved swab sample.** No vortex. Transfer to a 2.0 ml **bead microtube** containing particles.

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 **50 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

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