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DANAGENE MICROBIOME SALIVA DNA KIT

Ref.0623 50 Preps

1. INTRODUCTION

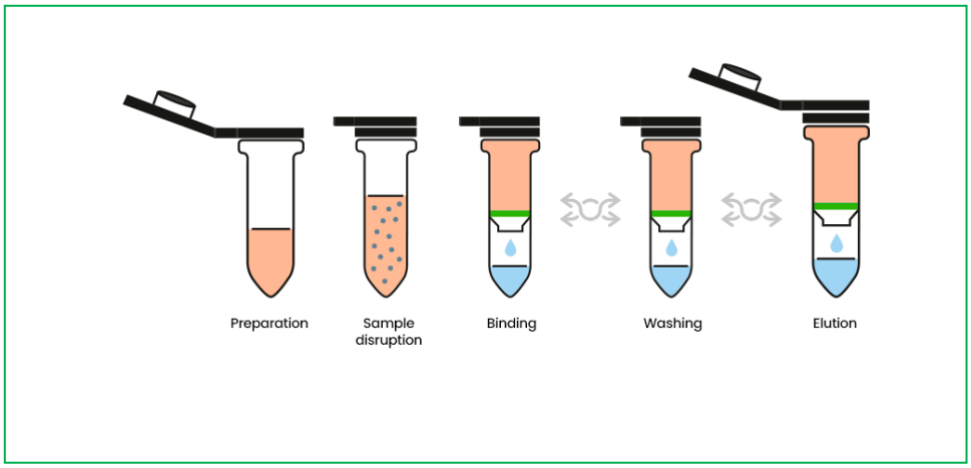
The **oral microbiome** is one of the most diverse of any human-associated microbial community. The oral microbiome is a causative factor in conditions such as dental caries, periodontal disease, and halitosis, and has also been implicated as a reservoir for infection at other body sites and in the pathogenesis of non-oral diseases, such as inflammatory bowel disease.

The DANAGENE MICROBIOME Saliva DNA kit has been designed for a fast and efficient purification of **microbial DNA** for microbiome analysis using:

a) **up to 600-800 µl of fresh saliva samples.**
b) **Preserved saliva samples with our DANASALIVA Sample Collection MICROBIOME Kit.**

Features:

- Designed for rapid purification of highly pure microbial DNA for microbiome analysis.
- Silica-membrane technology with MiniSpin columns.
- Bead Microtubes for efficient lysis included in combination liquid Proteinase K.
- Sample material: saliva / preserved saliva samples.
- Typical yield: Approx. 2-20 µg depends on patient.
- Preparation Time: 35 min.
- Elution volume: 100 µl.



Beginning with a bead-beating protocol, cells are lysed through a combination of mechanical force, heat and detergent, vortexed using horizontal adapter for the Vortex Genie 2 Vortex or using others common disruption devices.

Appropriate DNA binding conditions to the Microbial DNA Columns are achieved by addition of large amounts of chaotropic salts (Binding Buffer) to the lysate. Contaminants are removed by two efficient washing steps. Afterwards, The resulting DNA is recovered in a DNA-free Tris buffer to use for subsequent reactions.

Microbial composition of saliva sample preserved at room temperature is unchanged after two months with DANASALIVA Sample Collection MICROBIOME Kit . Saliva samples were taken using our system and stored at room temperature. They were sampled at the indicated time points and processed with the DANAGENE MICROBIOME SALIVA DNA Kit. The isolated DNA was the subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Samples had a constant microbial composition.Fig.1

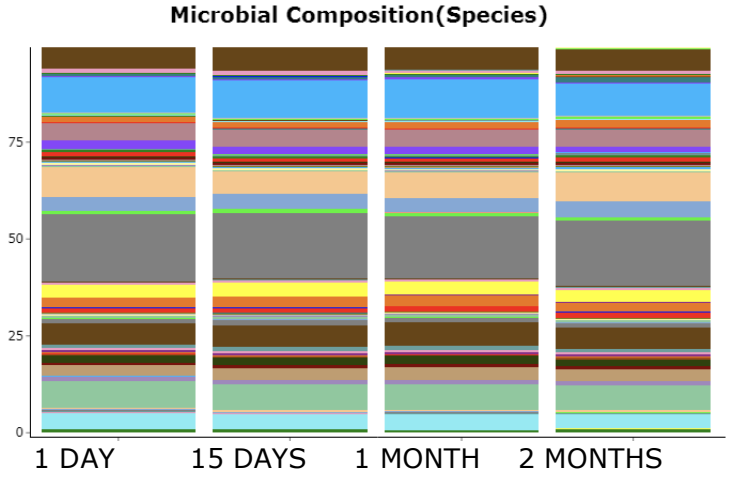


Fig.1 Saliva Samples with DANASALIVA Sample Collection MICROBIOME Kit -Species

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

- Saliva samples must be fresh or preserved saliva samples with our DANASALIVA Sample Collection MICROBIOME Kit.

- 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.

- 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).

- 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.

- In addition to the standard method, several modifications are possible to increase yield, concentration, and convenience.
  Convenient elution (standard elution): For convenience, elution can be performed by one-time addition of 100 µL elution buffer onto the column.
  High yield: Two serial elutions of 100 µL each for total elution volume of 200 µL.
  High concentration: Use initial 100 µL eluate for second elution – 100 µL total elution volume, 2 elutions.

3.3 Protocol for microbial DNA isolation from saliva samples

a)Saliva samples*: Centrifuge **600-800 µl saliva sample** for 90 seconds. Remove the supernatant using a pipette and avoiding damaging the cell visible white pellet.

b) Preserved saliva samples in DANASALIVA Sample Collection Kit*:
1b. In the DANASALIVA Sample Collection MICROBIOME Kit it will be appreciated a white pellet containing the buccal cells. Shake the tube containing 2 ml of the collected saliva. **It is important to observe a homogeneous solution before to take 1.2 ml of sample.**

2b. Centrifuge **1.2 ml (saliva + saliva preservation solution)** for 90 seconds at 13.000-16.000 x g. Remove the supernatant using a pipette and avoiding damaging the cell visible white pellet. Re-centrifuge (spin pulse) and eliminates the total liquid.

** If the cell pellet is very small you can add another saliva sample to obtain bigger a pellet in order to obtain more DNA yield and repeat the centrifugation step*.

1. Add **850 µL of Microbiome Lysis Buffer** to the cell visible white pellet. Resuspend the pellet using a micropipette. 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 **100 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