REAL MICROBIOME SALIVA DNA KIT

Ref. RBMEGS19 50 Preps

1. INTRODUCTION

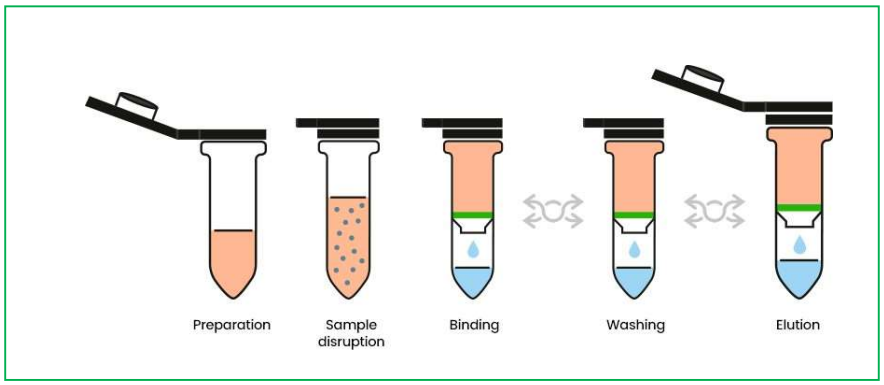
The **oral microbiome** is one of the most diverse of any human-associated microbial community . The oral microbiome is a causative factor in conditions such as dental caries , periodontal disease , and halitosis , and has also been implicated as a reservoir for infection at other body sites and in the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

REAL 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 REAL 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 REAL SALIVA 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 REAL MICROBIOME SALIVA DNA Kit. The extrated DNA was the subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Samples had a constant microbial composition.Fig.1

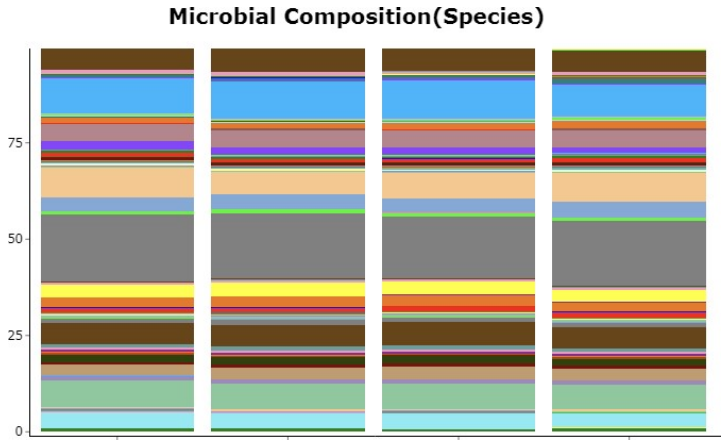


Fig.1 Saliva Samples with REAL SALIVA Sample Collection MICROBIOME Kit -Species

2. KIT COMPONENTS

	50 Preps	Storage
CTAB Extraction Buffer	**45 ml**	Room Temperature
Binding Buffer	**15 ml**	Room Temperature
Desinhibition Buffer*	**18 ml**	Room Temperature
Wash Buffer *	**10 ml**	Temperatura ambiente
Elution Buffer	**10 ml**	Temperatura ambiente
Bead Microtubes	**50 units**	Temperatura ambiente
Proteinase K*	**30 mg**	-20ºC
Microbial DNA Columns	**50 units**	Temperatura ambiente
Collection Tubes	**100 units**	Temperatura ambiente

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

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 (70°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 10 ml of Ethanol 100 %** to the Desinhibition Buffer. Keep the container closed to avoid the ethanol evaporation.

- **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

- Saliva samples must be fresh or preserved saliva samples with our REAL 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.

- 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 REAL SALIVA Sample Collection Kit*:
1b. In the REALSALIVA Sample Collection MICROBIOME Kit it will be appreciated a white pellet containing the buccal cells .Shake the tube containing 1 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 eliminate the total liquid.

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

1. Add **800 µl of CTAB Extraction Buffer** to the cell visible white pellet. Resuspend the pellet using a micropipette. No vortex.

2. **Add 25 µl of Proteinase K. Incubate at 70ºC 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 for5 minutes**.

5. Transfer up to 500 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 **250 µl of Binding Buffer** and vortex briefly.

7. Load the sample mixture onto a **Microbial DNA columns-tube assembly**, and Discard the flow-through.
   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.

8. Place the Microbial DNA column in a clean collection tube, add **500 µl of Desinhibition Buffer.Centrifuge at 14.000 rpm for 1 minute.** Discard the flow-through.

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

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

11. 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. Incubate **at room temperature** for **2 minutes**.

12. **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: durviz@durviz.com