REAL SWABS DNA KIT

Ref. RBMEG20 100 Preps
Ref. RBMEG21 500 Preps
Ref. RBMEG22 1000 Preps

1. INTRODUCTION

DANAGENE SWABS DNA Kit provides a method for the extraction of high quality genomic **DNA from buccal swab samples with foam brushes and saliva samples collected with our REALSWABS Sample Collection Kit.**

It is a fast, safe and economical method. It uses a step of deproteinization with a novel saline buffer avoiding the use of toxic organic solvents such as phenol or chloroform.

In the case of saliva, obtaining DNA in the same individual presents an intrasubject variability, that is, there is not an approximate amount of DNA / ml since it can vary according to the time of collection of the sample, given the case of that a large amount of DNA is not obtained using swabs and subsequent applications that require a large amount of DNA can not be carried out.

	Ref. RBMEG20	Ref. RBMEG21	Ref. RBMEG22	
Resuspension Buffer	105 ml	2x 250 ml	2 x 500 ml	Room Temperature
Lysis Buffer	35 ml	305 ml	2 x 305 ml	Room Temperature
Protein Precipitation Buffer	22 ml	105 ml	2 x 105 ml	Room Temperature
DNA Hydrattaion Buffer	5ml	25 ml	50 ml	Room Temperature
Proteinase K	500 ul	2.50 ml	10 ml	- 20ºC
RNase	500 ul	2.50 ml	10 ml	-20ºC

Storage and stability

All components are stable for 12 months from the purchase date being stored as indicated

Equipment and additional reagents

- ✓ Isopropanol.
- ✓ 70%Ethanol.
- ✓ 1.5 ml and 2.0 ml microtubes.
- ✓ Microcentrifuge or clinic centrifuge.
- ✓ Vortex.
- ✓ Water bath.

3. PROTOCOL

3.1 Preliminary Preparations

SAMPLE TAKING

1. It is recommended that the individual to be sampled should refrain from drinking coffee and taking any food at least 30 minutes before collection. If this is not possible, a gentle wash with water from the mouth is recommended.

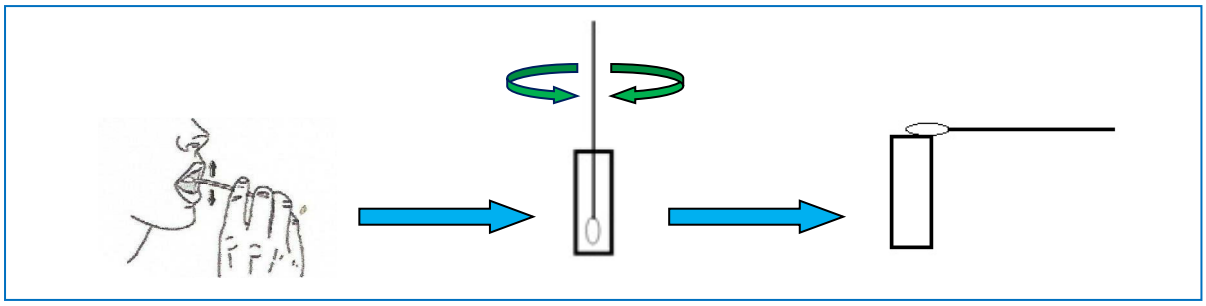
2. Collect the buccal cell sample with the foam swab. Rub the swab on the inside of the cheek (buccal wall) and gums with a firm pressure about 30 times on each side of the face and each side of the swab.

Important: make a firm, solid and reasonable pressure with the swab. The hand can be placed on the cheek to offer a more solid surface.

3. Proceed with the extraction or with the conservation method indicated according to the swab used.

4. If you use our REALSWABS Sample Collection Kit, proceed as follows:

5. Insert the swab into the microtube with the **Conservation Solution.** Rotate the swab quickly to release the buccal cells into the solution **with the same stirring motion of a teaspoon of coffee**. Press the swab against the wall of the microtube and rotate to ensure that most of the fluid remains in the microtube. It is also recommended to pass the foam head of the swab several times through the upper part of the microtube to release the last drops.



6. The solution should be observed that acquires a slight turbidity which will indicate the presence of oral cells. If the solution continues transparent, a new sample collection should be carried out with a new swab and at another time .

7. The preserved sample is stable at room temperature (15-25ºC) for 1 year and can be sent to the laboratory for the purification of genomic DNA. If stored at -20ºC or -80ºC, the sample is stable indefinitely.

3.2 Extraction from samples preserved with our REALSWABS Sample Collection Kit

1. Those samples that have been left upright for several days can be observed a white pellet containing buccal cells. Using a micropipette, resuspend the pellet completely and transfer **all the solution to a new 2.0 ml microtube.**

2. **Add 1000 µl of Resuspension Buffer or nuclease free-water** and Centrifuge at 13,000-16,000 x g for 2 minutes.

3. Remove the supernatant by decanting, **taking care not to lose the cell pellet.** Return to centrifuge briefly and remove all the liquid with a micropipette.

4. Add **300 µl of Lysis Buffer + 5 µl** de **Proteinase K** + **5 µl de RNasa** and resuspend the white pellet with micropipette. Incubate at 37ºC for 30 minutes. If it is possible vortex periodicaly .

5. Add **100 µl of Protein Precipitation Buffer.**

6. Vortex vigorously at máximum speed for 30 seconds. durante 30 segundos.

7. Centrifugate at 13.000-16.000 x g for 5 minutes.
It will be observed that the protein precipitate forms a very thin pellet (if there was little cellular pellet, sometimes the protein pellet is not observed).

8. Transfer the supernatant containing the DNA into a new 1.5 ml microtube containing **300 µl of isopropanol**. Mix turning the tube upside down.

9. Centrifugate at 13.000-16.000 x g for 3 minutes.

10. Remove the supernatant. Add **300 µl of ethanol 70%** to whash the DNA pellet.
Be careful not to lose the DNA pellet which will be very small or undetectable and will be more visible after washing 70% ethanol.

11. Centrifugate at 13.000-16.000 x g for 2 minutes. Carefully remove all the ethanol. Be careful not to lose the DNA pellet, which will be very small or undetectable.

12. Turn the microtube upside down on absorbent paper and leave it to dry for about 5 minutes.
You can also make a pulse with a centrifuge and remove the liquid with a micropipette.

13. Add **30-40 µl of DNA Hydratation Buffer** to ADN and resuspend with micropipette.

14. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

3.3 Protocol for DNA isolation from buccal swabs

The use of foam brushes such as those provided are adequate since they allow recovering almost all of the lysis buffer after the incubation period.

Cell lysis

1. Add **600 µl of Lisis Buffer** in a 1.5 ml microtube. Cutting the brush head and handle some filling into the microtube. Vortex vigorously to release the cells from the brush.

2. Add **5 µl** of **Proteinase K (20 mg/ml)** + **5 µl of RNase** . Mix.

3. **Incubate at 37ºC for 60 minutes.** If it is possible vortex periodically .

4. Remove the brush head of the lysis solution, rubbing against the walls to collect the maximum amount of liquid.

Protein precipitation.

1. Add **200 µl of Protein precipitation solution** to the cell lysate.

2. Vortex vigorously at maximum speed for 20-30 seconds.

3. Centrifuge at 13.000-16.000 x g for 3-5 minutes. A white precipitate will be formed.

DNA precipitation.

1. Transfer the supernatant containing the DNA into a new microtube containing **600 µl of Isopropanol**.

2. Mix turning the tube upside down for about 50 times.

3. Centrifuge at 13.000-16.000 x g for 2 minutes. The DNA will be visible as a white pellet.

4. Remove the supernatant and dry the tube on absorbent paper. Add **600 µl of Ethanol 70%** to wash the DNA.
Be careful not to lose the DNA pellet which will be very small or undetectable and will be more visible after washing 70% ethanol.

5. Centrifuge at 13.000-16.000 x g for 1 minute. Carefully remove the supernatant, avoiding touching the DNA pellet. It can be briefly centrifuged to collect the drops of residual ethanol.

6. Turn the microtube upside down on absorbent paper and leave it to dry for about 5 minutes.

DNA Hydratation
1. **Add 30-40 µl of DNA Hydratation Solution** and mix well with pipette.

2. Keep it at 2-8ºC. For long-term storage, store at -20ºC/ -80ºC.

4. PROBLEM GUIDE AND POSSIBLE ANSWER

For any doubts or additional questions about the protocol, please contact the technical service of DURVIZ S.L.